

Effects of canopy manipulation and environment on carbon resource allocation to flowering and fruit set in apple

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**This thesis is submitted in partial fulfilment of the requirement for the degree of Doctor of Philosophy at
the University of Tasmania**

**Havelock North
April 2016**

Statements and Declarations

Statement of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of the background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

Kenneth Charles Breen. April 2016.

Authority of Access

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Statement of Co-Authorship

Chapters two through five are co-authored papers or manuscripts. The name, affiliation, role and contribution of each of the colleagues to each paper or manuscript is noted below.

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The Candidate was the primary author, managed all data collection and entry, and conducted all statistical analysis. All authors contributed to refinement and presentation of the final paper. In addition, author 2 contributed significantly to development of the field methodology and data collection, and author 4 advised and assisted with methodology for statistical analysis and development of models.

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Paper 4, Chapter 5. A re-evaluation of the role of carbohydrate reserves in fruit set and early season growth of apple

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The Candidate was the primary author, managed all data collection and entry, and conducted all statistical analysis. All authors contributed to refinement and presentation of the final paper. In addition, author 2 contributed to the idea, and author 4 conducted carbohydrate analyses after initial sample preparation by the candidate.

The percentages following each colleague listed below are indicative of their overall contribution to the body of work presented in this thesis.

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In this programme, study sites were distributed across countries and situated up to 2800 km from 'home'. To ensure that trees were managed properly and data was collected timeously, I have relied on the assistance of colleagues working with me in the PIPS program as well as at PFR sites. I have really appreciated the help of John Wilkie, Heidi Parkes and Osi Tabing (Applethorpe Research Station, DAF Queensland); Sally Bound (Tasmanian Institute of Agriculture, University of Tasmania); Murray Oliver, Daya Dayatilake, and Ben van Hooijdonk (Plant & Food Research, Hawke's Bay) and Rob Diack, Shona Seymour and Angie Shirtliff (Plant & Food Research, Riwaka).

Trees used in this research were situated on four commercial orchards and one research orchard. I am indebted to the owners and managers of these orchards for allowing me to completely change the structure of some of their trees in the process of this work and to cause a few headaches while we merged research requirements with orchard management requirements. Richard Lyons (Apollo Pac/Turners and Growers, Hawke's Bay, New Zealand), Maurice Silverstein (Shepparton, Victoria), Daniel Nicoletti (Stanthorpe, Queensland), Scott Price (Calvert Bros, Huon Valley, Tasmania), Sam Stringer (Plant & Food Research, Motueka).

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A number of colleagues in Plant and Food Research, reviewers for the journals *Acta Horticulturae* (<http://www.actahort.org/>) and *Scientia Horticulturae* (<http://www.journals.elsevier.com/scientia-horticulturae>), and examiners of this thesis have made valuable suggestions that have undoubtedly improved the quality of the final version. Their time and insight is greatly appreciated.

I hope that I have not bored my friends with long answers to short questions. Thank you for listening, and Cris Sanders for going beyond the call of duty to help remove buds one day in 2012.

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Christianity is the foundation of my life. Unsurprisingly, for me this adventure has been about spiritual understanding as much as scientific knowledge. Thank you Lord for revealing some of the wonders that you hold.

Abstract

In commercial apple production, variability in yield and fruit quality among trees, orchards and seasons may have significant negative impact on profitability of individual orchards, and ultimately, the global competitiveness of the national industry. Although the Australian and New Zealand apple industries differ widely in many aspects, they both recognise the need for increased production of high quality fruit in order to expand export markets.

In spring, competition among flowers, developing fruit, and shoots for limited resource supply induces fruitlet abscission, reduces potential fruit size, and may negatively affect fruit quality and induce biennial bearing. In commercial apple production, natural flowering and fruit set generally result in crop loads that are too high to achieve premium fruit size and quality targets. Orchardists seek to reduce flower and fruit numbers to their commercial target as soon as possible in spring. However, current standard technology relies on chemical thinning which does not give reliable or predictable responses. Apart from increasing risk, this leads to delays in achieving final fruit numbers, which results in loss of potential fruit size and quality. The mechanisms regulating flowering, fruit set and abscission in perennial crops such as apple are complicated and not fully understood. Improved knowledge of the physiology of these processes is required if commercial thinning practices are to be improved and consistently high yields of high quality fruit are to be achieved.

Artificial spur extinction (ASE) provides a useful tool to investigate the physiology of flowering, fruit set and abscission. ASE is a crop load management tool that uses hand-thinning of whole buds in late dormancy to set targeted floral bud densities consistently on every limb. The process retains buds on spurs and short shoots in well illuminated areas of the canopy and spaces them evenly along the limb. Chemical thinning is not used. In this thesis, removal of floral and vegetative sinks as well as potential leaf area (carbohydrate source) using ASE, provided a means to investigate the role of carbohydrate source-sink relationships in annual flowering and fruit set. The research was conducted on 'Gala' strains, in five different commercial production environments through Australia and New Zealand over four seasons. This allowed examination of influence of environment on flowering and fruit set in this genotype.

This thesis is presented in six chapters. The first provides background to the investigation, identifies the primary hypotheses tested and gives an outline to the thesis structure. The second to fifth chapters describe specific investigations that were undertaken and discuss the results in the context

of current scientific knowledge and commercial technology. The last chapter provides a summary of outcomes, and, with a general discussion of the conclusions arrived at in the investigative research, discusses the relevance of the outcomes of the research to scientific knowledge and commercial technology.

In the first research chapter, trees managed using ASE were compared with trees thinned after final fruit drop (Control) on five sites through New Zealand and Australia over four seasons. In Control trees flowering and fruit set was unpredictable and highly variable among trees within sites, among sites, and between seasons. Reducing floral bud density using ASE greatly reduced variability in flowering and fruit set and allowed predictive fruit set models to be developed for each site. Results led to investigations of the role of carbohydrates in fruit set and development during the early part of the season. These investigations are discussed in the ensuing three research chapters.

Using two treatments, ASE and flower cluster thinning before bloom (FCT), and comparing these treatments with trees in which flower numbers were not altered (Control), allowed investigation of the effects of early sink removal (ASE and FCT vs Control), and altered leaf area and access to stored carbohydrates (ASE vs FCT) on fruit set. The effects of these factors on fruit development were also investigated by ensuring all treatments had the same final crop load, using post fruit drop hand-thinning to set either 4 or 6 fruit per cm² branch basal cross-sectional area (BCA). Reduction of floral bud density through FCT or ASE increased within-bud fruit set and led to greater harvest mean fruit weight. These results lead to a hypothesis that these treatments improved carbon availability within floral spurs during early-season development, and that the means by which this occurred differed between ASE and FCT. In FCT, reducing the density of flower clusters (sink size) without altering leaf area may have increased the availability of newly synthesised carbon to remaining sinks, improving fruit set and development. In ASE, removal of, and uniform spatial distribution of remaining buds may have improved irradiance of remaining fruiting spurs, thereby increasing photosynthate availability to the developing fruit within the spurs. These conclusions were examined in investigations on seasonal light interception and the role of stored carbohydrates in ASE canopies, which are discussed in chapters four and five.

ASE could be thought likely to reduce total early-season leaf area and light interception, as it greatly reduces total bud numbers on the tree. Fractional light interception was measured on ASE and Unmodified trees from shortly after dormancy, through one whole season. Where ASE was set at bud

densities producing commercial crop loads (4 and 6 buds per cm² BCA), early season light interception was not affected (6 buds per cm² BCA) or only marginally reduced (-2%, 4 buds per cm² BCA) very early in the season, but increased (+4%) over most of the season. In ASE managed trees, increased irradiance of remaining shoots, which are closely associated with flowers and developing fruit, was thought to lead to greater within-bud fruit set. Early removal of floral clusters in ASE managed trees is likely to have reduced competition for carbon resources during early fruit development thereby increasing harvest mean fruit weight. However, increased fruit weight may also have occurred through greater development of bourse shoots on ASE canopies increasing carbon supply to developing fruit later in the season.

The contribution of carbohydrate reserves to fruit set in apple is unclear. In canopies where ASE is imposed, reduced competition among developing buds for limited carbohydrate reserves may contribute to increased within-bud fruit set compared with unmodified canopies. Early post-harvest defoliation was used to manipulate carbohydrate reserve concentration and investigate the effect on changes in carbohydrate concentration and fruit set the following spring. Carbohydrate concentration in roots, shoots and spurs showed that reducing reserve carbohydrate concentration in these tissues had very little direct impact on fruit set the following season. This led to the conclusion that newly synthesised photosynthates play a much greater role than reserves in supplying carbohydrate to young flowers and fruit, and consequently play a greater role in determining fruit set.

Increased within-bud fruit set and improved harvest fruit weight observed in ASE treatments in this series of studies support the hypothesis that removal of competing sinks in late dormancy improved availability of limited carbohydrate resources to remaining sinks. However, contribution from newly synthesised carbohydrates in early spring seemed to play a much greater role in fruit set than availability of stored carbohydrates that were re-mobilised in spring. Consequently, light interception by the canopy, and in particular illumination of leaves closely associated with fruit (e.g. on the same shoot), are likely to play a large role in within-bud fruit set and fruit development; a concept supported in the literature. This might suggest that as many buds should be retained in the canopy as possible in order to maximise early-season canopy light interception. However, on the contrary, this thesis showed that even when about 50% of buds were removed using ASE, whole canopy light interception was increased the following season. Because increased fruit set and fruit weight were also observed in ASE treatments, it was concluded that improved irradiance of leaves on remaining fruit-bearing

shoots in ASE more than compensated for the reduced early spring leaf area over the whole canopy. Highly variable fruit set and loss of potential size and fruit quality are a significant limitation in current commercial crop loading technologies, which rely on chemical thinning. Removal of floral sinks and improved irradiance following ASE treatment, greatly improved the reliability and predictability of fruit set, even in seasons where low light intensity, frost, or pollination may have reduced fruit set. Thus ASE provides a technology highly suited to replacing current commercial technologies and that is more likely to fit with sector aspirations of consistently reliable annual production of high quality fruit.

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Chapter 1. Introduction

Background

The Australian apple industry is facing a number of challenges. The sector is currently focussed on supplying the local market (O'Rourke, 2009) in which apple consumption has historically been low compared with similar nations (O'Rourke, 2008, 2015) and in competition for market share with other foods. In order to meet World Trade Organisation obligations, restrictions preventing importation of apples have recently been lifted, and growers expect this to result in further challenges in the local market through competition from imported apples (Apple & Pear Australia Limited and Horticulture Australia Limited, 2008, 2010b, a; O'Rourke, 2015). At present, this competition appears to be limited. On Australian orchards productivity is generally low (O'Rourke, 2009, 2015), labour costs are high compared with other nations, and drought and climate change create further uncertainty (Apple & Pear Australia Limited and Horticulture Australia Limited, 2010b).

In 2010, Apple and Pear Australia Ltd (APAL) approved the industry strategic plan to 2015. This plan recognised the challenges facing the industry and identified the need for improved fruit quality and increased production within sustainable parameters (given the challenges of limited water availability and changing climate), in order to expand export markets and stimulate domestic demand (Apple & Pear Australia Limited and Horticulture Australia Limited, 2010b). This plan already appears to have had positive impact, with consumption of fresh apples over the period 2012 – 14 increasing by ~70% per capita from 2002 – 04 (6.6 to 11.1 kg per annum) (O'Rourke, 2015).

The New Zealand industry differs appreciably from the Australian Industry. New Zealand apple productivity per hectare is amongst the highest in the world (Palmer et al., 2002; O'Rourke, 2015) and until recently, per capita consumption has been about twice that of Australia (O'Rourke, 2015). The sector relies on exporting product and is targeting NZ\$1 billion export earnings by 2022. A record NZ\$700 million export is expected in 2016, the third record season in a row (Freshplaza, 2016). Although New Zealand regularly lies in the top ten fresh apple exporters in the world, it generally produces only ~4% of global export production and relies on supplying premium fruit and new varieties to capture market share (O'Rourke, 2015).

Globalisation of the apple market has given producers advantage through opening of new markets, however, it has meant that producers must be more competitive, as buyers have more sources from which to acquire product (O'Rourke, 2003). In the apple industry, producers tend to compete through

increased productivity and/or higher average value (O'Rourke, 2015). In future, marginal growers and regions will become increasingly less competitive and remaining producers will rely on every available technology to increase efficiency and yield per hectare, and reduce unit costs (O'Rourke, 2003).

Production systems have come under increasing scrutiny as the “fear of food” phenomenon (consumer suspicion of the food industry despite minute occurrence of food-related ill-effects) has grown. This has been coupled with increasing demands by consumers for producers to be “sustainable” (O'Rourke, 2015). The term “sustainable” appears to be fairly loosely defined by consumers, but generally is viewed as having something to do with ecological sustainability.

Consequently, fresh food “quality” now not only has to do with visual attributes, taste, and texture, but also consumer perception of the system under which the food was produced. Apple producers in both Australia and New Zealand are competing in these markets.

Variability in production is strongly detrimental to production efficiency (O'Rourke, 2015). To ensure their financial sustainability and in order to achieve sector targets, consistently high fruit quality and yield targets must be achieved on individual orchards with little variation among seasons and trees. In order to accomplish this, natural variability in flowering and fruit set, must be effectively managed. In general, commercial apple trees produce many more flowers and fruit than are needed to optimise economic return, and flowers and fruit must be removed to achieve required fruit size and quality (Wertheim and Webster, 2005). Current technologies to manage flowering and fruit load are centred on chemical thinning. Although this technology is moderately effective, there are a number of challenges associated with its use, primarily that results are not sufficiently predictable, meaning that hand-thinning must also be employed (Greene and Costa, 2013), and there is loss of potential fruit size arising from delayed fruit removal (Jackson, 2003). Understanding of the physiology of flowering and fruit set in apple has improved substantially over the last few decades (Greene and Costa, 2013). However, the persistent lack of predictability of commercial thinning outcomes using current techniques is demonstration of the need for further knowledge of the physiology of flowering, fruit set and fruit development, as opposed to only research on development of new chemistry or technologies. The use of artificial spur extinction (ASE) provides a useful tool to investigate this physiology, but it is also interesting because it is an emerging technology in use for commercial crop load management. ASE uses hand-thinning of whole buds in late dormancy to reach targeted floral bud densities on every limb. This is achieved by measuring the cross-sectional area of the base of

every branch and multiplying that number by the density of buds required. This gives a number of buds that are required for that branch and excess buds are removed by hand. The process preferentially retains floral buds on spurs and in terminal positions on short shoots (referred to as a “terminal buds”) in well illuminated areas of the canopy and seeks to space them evenly along the limb (Figure 1). Because lateral buds on one-year-old shoots generally produce fruit of inferior quality, the ASE process generally completely removes these buds between late dormancy and flowering. On long shoots (>20cm), some de-blossomed lateral buds may be retained, in order to allow formation of additional flowering sites in the following season. Chemical thinning is not used. As ASE removes the whole bud i.e. floral and vegetative sinks as well as potential leaf area (carbohydrate source) prior to bud break, it provides a means to investigate the role of source-sink relationships during flowering and fruit set.

The term “fruit set” is often not well defined. In this thesis I have followed the terminology used by Tromp and Wertheim (2005) where fruit remaining after “June drop” (northern hemisphere) or “December drop” (southern hemisphere) are regarded as “fruit set” or “final fruit set”.



Figure 1. Top: A dormant 'Royal Gala' branch showing the three types of buds referred to in this text. One-year-old shoots (arrowed line) carry terminal buds (solid circles) on their distal ends and lateral buds (dotted circles) along their length. Spur buds (broken circles) are situated in terminal positions of short shoots. Bottom: The process of artificial spur extinction applied to the 'Royal Gala' branch above completely removes buds (circles) in late dormancy. The number of buds to be retained on each branch is determined by the branch basal cross-sectional area (in this case 2 cm^2) and the density required (in this case 5 buds.cm^{-2}) to retain floral buds on spurs and in terminal positions (in this case 10 buds) on short shoots in well illuminated areas of the canopy spaced evenly along the limb.

Hypotheses and Objectives

Fruit set and early fruit development (leading to final fruit size and yield) rely on supply of carbohydrates and nitrogenous compounds which are sourced from reserves and current photosynthesis. The relative importance of these sources is not well understood (Oliveira and Priestley, 1988; Tromp, 2005). During the period 2 – 4 weeks after bloom, carbohydrate demand from developing shoot and fruit sinks is likely to be greater than supply, and shoot development is thought to have priority for limited carbohydrate supply over fruit development. This competition among sinks is believed to result in fruit abscission (Lakso et al., 1999; Greene et al., 2005; Lakso et al., 2006). Fruit set is dependent on the presence of primary spur leaves, and reduction in primary spur leaf area negatively affects fruit set (Ferree and Palmer, 1982).

In order to improve understanding of the physiology of flowering and fruit set, this PhD examined two hypotheses:

- 1) Reducing the number of floral and vegetative sinks early in the season will reduce competition among remaining sinks for stored reserves and newly synthesised photosynthates, and result in increased fruit set.
- 2) Removal of whole buds, for example through ASE, will result in impaired fruit set because reduced early season spur leaf area (carbohydrate source) will reduce light interception by the canopy.

Increased understanding of the physiology of flowering and fruit set of apple resulting from this work was aimed at improving technologies for commercial apple production in Australia and New Zealand.

Thesis structure

Doctoral candidates at the University of Tasmania are encouraged to present their thesis in the form of a series of chapters which individually have been, or will be submitted for publication to peer reviewed scientific journals. This format has been followed in this thesis. Details of the relative contribution of each author to each paper are presented in the “Statement of Co-Authorship” above. The journal to which the paper was submitted and its publication status at the time of submission of the thesis are presented in “Details of Publication of Research Papers” below. In some cases, part of

the data in each paper were presented in other forms such as client reports or oral conference presentations. These details are also presented in “Details of Publication of Research Papers”. The chapter structure of the thesis is as follows.

Chapter 2. Effects of environment and floral intensity on fruit set behaviour and annual flowering in apple.

The physiology of fruit set and its interaction with floral intensity and environment is not well understood and this inhibits more effective commercial crop load management sought by commercial apple producers. Flowering intensity of ‘Gala’ trees was altered using ASE on five sites through New Zealand and Australia over four seasons. Using a series of bud densities and naturally occurring differences in weather and orchard environment among regions and seasons allowed investigation of the effect of floral intensity and environment on fruit set and annual flowering. Flowering and fruit set in Control treatments was typical of commercial trees; highly variable, highly responsive to environmental conditions prevalent during fruit set, and largely unresponsive to differences in floral bud density. Reduction of floral bud density using ASE greatly reduced variability in fruit set and flowering and allowed predictive models to be developed for each site describing the response of fruit set to floral bud density. In the following chapters, the physiology affecting these responses was investigated.

Chapter 3. Method of manipulating floral bud density affects fruit set responses in apple.

Research in this chapter was undertaken to investigate the influence of early season carbohydrate source size (specifically leaf area and access to stored carbohydrates) and sink size (specifically floral and vegetative bud number) on fruit set and development. Artificial spur extinction (ASE) was used as a tool to manipulate sink size through complete removal of whole buds during dormancy. This likely reduced early season leaf area while improving access of remaining sinks to stored carbohydrates. Flower cluster thinning (FCT) prior to bloom reduced floral sink size without altering vegetative sink size or leaf area. These treatments were compared with an unmodified Control. Results imply that compared with the Control, manipulation of floral bud number through FCT or ASE increased carbon availability within floral spurs during early-season development. However the process by which this occurred appeared to differ between FCT and ASE. In FCT, reducing the

density of flower clusters (sink size) may have increased carbon availability to remaining sinks, improving fruit set and development. In ASE, removal and uniform spatial distribution of buds may have improved irradiance of fruiting spurs, thereby increasing photosynthate availability to developing fruit within the spur. These conclusions were investigated through examining the development of light interception and role of stored carbohydrates in ASE canopies which are discussed in chapters four and five.

Chapter 4. Artificial spur extinction alters light interception by 'Royal Gala' apple trees.

Reduction of early season bud density using ASE appears to increase carbon availability within remaining floral spurs because ASE management results in increased within-bud fruit set and improved fruit development. However, because ASE greatly reduces total bud numbers on the tree it is likely to reduce total early-season leaf area and thus reduce light interception and photosynthetic potential. Using ASE, a series of bud densities were imposed on 'Royal Gala' canopies in a commercial orchard in New Zealand and compared with unmodified trees. Fractional light interception was measured at intervals from shortly after bud break, through one whole season. Before petal fall, at ASE bud densities of 4 and 6 buds.cm⁻² branch basal cross-sectional area, which achieved commercial crop loads, early season light interception was either not affected (ASE6), or only slightly reduced (~2%, ASE4) compared with unmodified trees. During most of the season, light interception by ASE canopies was greater than unmodified canopies. Greater development of bourses and bourse shoots on ASE canopies probably increased light interception by shoots closely associated with flowers and developing fruit, increasing harvest mean fruit weight.

Chapter 5. A re-evaluation of the role of carbohydrate reserves in fruit set and early season growth of apple.

Carbohydrate reserves are considered essential in supporting early spring growth, however their contribution to fruit set is unclear. In canopies where ASE was imposed, reduced competition among developing buds for limited carbohydrate reserves may contribute to an increase in within-bud fruit set compared with unmodified canopies. In ASE and unmodified trees with high yields, carbohydrate reserve concentration in winter was manipulated through early defoliation shortly after harvest in the previous season and compared with carbohydrate reserve concentration in trees where natural

defoliation occurred. Carbohydrate concentration in roots, shoots and spurs was determined the following season from dormancy through to just after final fruit set. Results showed that differences in reserve carbohydrate concentration when growth resumed in spring had no direct influence on fruit set, and fruit set appeared to be more reliant on the availability of newly synthesised photosynthates. However, bourse shoot development was enhanced in treatments which had unmodified reserve concentration and reduced sink demand induced by ASE. Stored carbohydrates appeared to be utilised in the very early development of vegetative sinks and may promote canopy development in spring

Chapter 6. Summary and implications.

This chapter summarises the outcomes of the research and discusses the conclusions arrived at in the context of the original objectives of the work and the hypotheses tested. The relevance and implications for scientific knowledge and commercial production are also considered.

Details of Publication of Research Papers

Chapter 2. Effects of environment and floral intensity on fruit set behaviour and annual flowering in apple.

This chapter is published in *Scientia Horticulturae* (Breen et al., 2016).

Portions of the flowering and fruit set data, excluding modelling were presented in other forums:

Flowering and fruit set data for 2010 in Tasmania, Victoria and Queensland were presented to the “Horticulture for the Future” conference of the joint APHC/AuSHS/NZIAHS meeting in Lorne, Australia in September 2011 (Breen et al., 2011).

Flowering and fruit set data for 2010 and 2011 at all sites were presented as an oral paper to the 10th International Symposium on Orchard Systems in Stellenbosch, South Africa in December 2012 and are published in the proceedings of that Symposium (Breen et al., 2014b).

This research was funded in part by Horticulture Innovation Australia Ltd (HIA) as part of the apple and pear industry Productivity Irrigation Pests and Soils (PIPS) flagship program. Flowering and fruit set data for all Australian sites for all seasons were presented in the final report to HIA (Breen et al., 2014a).

Chapter 3. Method of manipulating floral bud density affects fruit set responses in apple.

This chapter is published in *Scientia Horticulturae* (Breen et al., 2015).

Chapter 4. Artificial spur extinction alters light interception by ‘Royal Gala’ apple trees.

This chapter was presented as an oral paper to the Symposium on Physiology of Perennial Fruit Crops and Production Systems at the 29th International Horticultural Congress in Brisbane, Australia in August 2014, and has been accepted for publication in the proceedings of that Congress (Breen et al., in press).

Chapter 5. A re-evaluation of the role of carbohydrate reserves in fruit set and early season growth of apple.

This chapter is in preparation for submission to the journal ‘Tree Physiology’.

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Chapter 2. Effects of environment and floral intensity on fruit set behaviour and annual flowering in apple

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Abstract

Natural variability in flowering and fruit set of commercial apple trees (*Malus x domestica* Borkh.) must be effectively managed to achieve optimised yield and quality. Early removal of excess flowers and fruit improves fruit size and quality. However, physiological responses to environment and flowering intensity, and their interaction with chemical thinning are not well understood, causing unpredictable fruit set responses. We altered flowering intensity on 'Gala' trees on five sites through New Zealand and Australia over four consecutive seasons using artificial spur extinction (ASE) at bud densities of 2 to 6 buds.cm⁻² branch basal cross-sectional area. We compared this with natural flowering and fruit set on trees thinned by hand after final fruit set (Control). ASE uses hand-thinning of dormant buds to specific densities in late winter, in order to reach targeted floral bud densities on every branch on whole trees the following spring. Naturally occurring seasonal differences in weather and orchard environment across five regions (Hawke's Bay and Nelson in New Zealand and Queensland, Tasmania and Victoria in Australia) allowed us to investigate the effect of these factors on fruit set and annual flowering behaviour. Typical of trees in commercial production, flowering and fruit set in

Control trees differed widely among trees within sites, among sites, and between seasons. Natural fruit set in these trees, especially at high floral bud densities, was highly variable and largely unresponsive to natural differences in floral bud density, but often highly responsive to differences in weather and other environmental conditions. If only one fruit was set per bud, the number of flowering buds was always sufficient to produce a commercial crop, but in some cases four times more floral buds were present than required. Systematic removal of dormant buds using ASE reduced floral bud density to within 20 - 30% of the bud density set during dormancy, and reduced variability in floral bud density among trees to about half of that in Control trees. In ASE trees, reduced floral bud density resulted in reduced number of fruit set per cm² branch cross-sectional area, but increased fruit set per floral bud because lower proportions of floral buds set zero fruit and greater proportions set two-or-more fruit, compared with Control trees. The ASE treatments allowed models to be fitted for each site which described relationships between fruit set and floral bud density, and identified years where weather or environment altered fruit set responses. Consequently, use of ASE in 'Gala' provided a method of very early crop load adjustment which produced reliable and predictable outcomes to fruit set over a number of sites and seasons.

Keywords

Thinning; artificial spur extinction; model; crop load; biennial bearing

Introduction

In commercial apple production, crop load must be managed in order to maximise economic return. Large crop loads are attractive from the point of view of increased yield, however, they may lead to branch breakage, reduced cold hardiness, and induction of irregular bearing (Dennis, 2000). Both excessively light and heavy crop loads reduce fruit quality and may result in fruit sizes that have lower consumer demand, thereby affecting market returns (Byers, 2003; Wertheim and Webster, 2005). The physiology and management of natural variability in flowering and fruit set and its relationship to crop load and yield of fruiting trees has been discussed for thousands of years. Both Theophrastus c.371-287 B.C. (Theophrastus, Trans. 1976) and Palladius in the first century A.D. (Palladius, Trans. 1807) discussed thinning of fruit during the season to improve the fruit to be harvested. Cotton

(1675), Langley (1728) and Lawson (1927) encouraged thinning of pome and stone fruit to ensure return bloom and to improve size or quality of remaining fruit.

In modern commercial apple orchards, trees normally produce far more flowers and fruit than are required for a commercial crop (Wertheim and Webster, 2005). On 'Royal Gala'/'M9' trees in commercial production in New Zealand, we have frequently calculated annual production of more than 3000 flowers, on 600 spur and terminal sites per tree. Flowering on one-year-old lateral buds may increase this to 4000 flowers, on trees where the required commercial crop loads are 250 to 300 fruit per tree.

Routinely, and even allowing for natural abscission, apple trees must have excess fruit removed in order to achieve production targets of fruit quality, size and regular bearing. In some high-value apple crops, only hand thinning of flowers and fruit is practiced, however this is usually considered uneconomic as it is labour intensive (Koike et al., 1998; Byers, 2003). Standard commercial practice relies on applying a series of chemical thinners through flowering and early fruit growth, followed by hand thinning after final fruit set (Wertheim and Webster, 2005). However, this practice has a number of shortfalls. Among these are loss of potential final fruit size (Jackson, 2003) possibly contributed to by use of products that thin through inhibition of photosynthesis or cause leaf damage; risk of phytotoxic response, small or deformed fruit, and over-thinning (Dennis, 2000); incompatibility with broader orchard management practices such as integrated pest management, or perception by consumers that the chemicals used have negative effects to human and environmental health (Dennis, 2000; Byers, 2003). Greater understanding of the mode of action of chemical thinners and the development of models to predict their effect has improved predictability of the result following their use in some environments. However, much of the physiology and interaction of chemistry, biology and environment remains unclear. This results in unpredictable outcomes and prevents more effective use of chemical thinners (Robinson and Lakso, 2011; Greene and Costa, 2013).

Development of the self-thinning trait in apples may reduce growers' reliance on chemical thinning in future, however this characteristic is not common in apple (Celton et al., 2014). Mechanical blossom thinning is in the early stages of commercial use, however there are concerns over damage and removal of spur leaves resulting in small fruit size and disease infection (Greene and Costa, 2013). Artificial spur extinction (ASE) is in commercial use in France (Lauri et al., 2004) and in the early stages of commercial use in New Zealand. The ASE process thins buds on whole trees during late

dormancy to the same densities as the required final density of fruiting buds. This crop load management technique has been shown to reduce biennial flowering and have a high degree of predictability of fruit set without the need for chemical thinning (Tustin et al., 2012; Breen et al., 2014). Our knowledge of the mechanism of floral induction and bud formation in perennial trees such as apple is also lacking (Tromp, 2005; Bangerth, 2006). Understanding these processes is difficult, primarily because the period of floral initiation and bud formation occurs over almost one year, during which many biotic and abiotic factors may act on the process. Research in this area has often been aimed at finding horticultural solutions to problems, rather than physiological understanding (Jackson, 2003; Tromp, 2005). Although flowering intensity in commercial orchards is not usually deficient, a number of factors may affect variability and intensity of flowering and fruit set among seasons, trees, and within trees. The presence of developing seeds in fruit can prevent floral bud development in spurs arising from the bourse and may induce irregular bearing both among seasons and among canopies. This may be further influenced by a number of abiotic factors (Chan and Cain, 1967; Monselise and Goldschmidt, 1982). Frosts of -2 to -3 °C are capable of killing apple buds, flowers and developing fruit (Proebsting and Mills, 1978; Lipa et al., 2008). Drought and waterlogging induce stomatal closure and reduce photosynthesis, and may result in reduced shoot growth, fruit set and flower bud development (Landsberg and Jones, 1981; Olien, 1987; Jackson, 2003). Pollination and fertilisation are also affected by weather (affecting pollinator activity and pollen germination and growth), pollen source, and pollen quantity (affecting the number of flowers and ovules pollinated) (Vicens and Bosch, 2000; Tromp and Wertheim, 2005; Wertheim and Schmidt, 2005). Conditions that reduce carbohydrate availability for early fruit growth increase fruit abscission. Cloudy periods may reduce light incidence, reducing photosynthesis and leading to lower carbohydrate status. When this occurs during high demand in the two to four weeks after bloom, and especially combined with high night temperatures which increase respiration, fruit drop increases (Lakso et al., 1999; Lakso et al., 2006b). Shading is known to inhibit floral bud formation within the canopy of apple trees (Cain, 1973). To improve understanding of the interaction of environment and floral intensity on the physiology of apple fruit set, we systematically manipulated floral intensity by dormant bud thinning using ASE, on trees in five regions through New Zealand and Australia, over four seasons. ASE was imposed over a range of bud densities and fruit set was investigated on both a branch basis and

flowering bud basis. Seasonal and site differences allowed us to investigate the effect of weather and orchard environment on fruit set and annual flowering behaviour.

Materials and Methods

This investigation was carried out on clones of 'Gala' apple trees grown on commercial orchards in five regions through New Zealand and Australia (Table. 1). Trees were mature, growing on semi-dwarfing to dwarfing rootstocks, and had been pruned to a Central Leader Tall Spindle tree management system. In each orchard in the winter prior to the first season of research, trees of similar canopy and trunk size were selected and allocated to five blocks, apart from the Nelson site which had four blocks. Within each block, single whole trees were randomly allocated to treatments using a randomised complete block design with two factors; Thinning and Fruiting bud density. The Thinning factor comprised artificial spur extinction (ASE) and post fruit drop hand thinning (Control, C). In the Fruiting bud density factor, treatments differed in the density of buds (between 2 and 6 buds.cm⁻² branch basal cross-sectional area (BCA)) that were allowed to retain fruit when trees were hand-thinned after final fruit set. Thus, ASE manipulated the density of floral buds by removing whole buds on individual branches in late dormancy. This was followed by hand thinning of individual fruit within buds after final fruit set, as described below. In the Control treatment, flowering was not manipulated, and hand thinning after final fruit set adjusted both the density of buds that held fruit (by removing all fruit on some buds), and the number of fruit that were retained within remaining buds, to match the corresponding ASE treatment (also described below). Each specific thinning treatment and fruiting bud density was applied to the same tree each season.

All trees were standardised in the following way. In winter, where necessary, branches were removed to leave six to seven branches per metre of effective canopy height. Remaining branches were tied down to 10 - 15° below the horizontal to reduce vigour. In most blocks, this required removal of numerous branches and removal of lower upward tending branches in the winter prior to beginning the research, but very little adjustment thereafter. Other than pruning and thinning, trees were managed according to the practices of each orchardist but all were under conventional commercial orchard management practices. Commercial yield targets for Australian orchards were 50 - 80 t.ha⁻¹, while those in New Zealand were 80 - 90 t.ha⁻¹. The sum of individual branch basal cross-sectional areas (BCAs) for each tree showed that at a fruiting bud density of four in Australia and five in New

Zealand, if each bud held one fruit, approximately the same number of fruit per tree would be achieved as the commercial targets for the orchards. These “commercial bud densities” were included in the series of ASE fruiting bud densities investigated in this study.

Table 1. Clones and orchard system details of ‘Gala’ apple used in this study. The investigation was conducted on trees situated at five sites through New Zealand (Hawke’s Bay and Nelson) and Australia (Queensland, Tasmania and Victoria).

Region	Site	Cultivar	Rootstock	Tree spacing (m)	Year planted
Hawke's Bay	Meeanee	'Royal Gala'	'M9'	3.4 x 1.25	2005
Nelson	Motueka	'Royal Gala'	'M9'	3.7 x 1.5	2006
Queensland	Stanthorpe	'Royal Gala'	'MM106'	4.8 x 1.8	2000
Tasmania	Huon Valley	'Royal Gala'	'M26'	4 x 1	2004
Victoria	Shepparton	'Galaxy'	'MM106'	4.5 x 2.0	1997

Between dormancy and early budbreak each season, ASE was applied individually on every branch through the ASE canopies. For each branch, the basal cross-sectional area was calculated and multiplied by the required bud density for that treatment to ascertain the number of buds to be retained on the branch. All other buds were removed by hand. In Australian sites, ASE bud densities of 3, 4 and 5 buds.cm⁻² BCA were applied over four seasons from 2010 - 2011 to 2013 - 2014. In New Zealand sites, ASE bud densities of 2, 3, 4, 5, and 6 buds.cm⁻² BCA were used in the first three seasons (2011 - 2012 to 2013 - 2014) and, to examine return bloom, 5 and 6 buds.cm⁻² BCA in the last season (2014 - 2015). Small buds, buds in shaded areas of the canopy, and those on the underneath of branches were preferentially removed, and retained buds were spaced out along the length of each branch. Where possible, terminal buds on short, 10 to 15 cm length shoots were kept, as these have been shown to produce larger fruit than other buds (Breen et al., 2007; Tustin et al., 2011b). One-year-old lateral buds were removed at all sites apart from Victoria.

After natural fruit drop was complete (end November) and fruit set had been recorded, ASE and Control treatments were hand thinned to their treatment-specific crop loads. For treatments in New

Zealand, ASE treatments set at 2 and 3 buds.cm⁻², were thinned to 4 fruit.cm⁻² BCA by leaving up to two fruit per bud. In all other ASE treatments at all sites, fruit were thinned to a single fruit per bud apart from occasions where two fruit per bud were required to compensate for buds that flowered, but failed to set fruit. Control trees were thinned so that they carried fruit (fruiting bud density) on 3, 4, or 5 buds.cm⁻² BCA in Australia, and 2, 4 or 6 buds.cm⁻² depending on site and season in New Zealand. The Control 2 treatment was thinned to carry 2 fruit per bud on 2 buds.cm⁻² (4 fruit.cm⁻² BCA). On all other Control treatments, fruit was thinned to a single fruit per bud, at treatment-specific fruiting bud densities, except where two fruit per bud were carried to compensate for some buds that flowered but did not set fruit.

No chemical thinners were applied to any treatments, allowing examination of the effects of Thinning and Fruiting bud density on flowering and fruit set. Flowering and fruit set were recorded on the same three representative mid-canopy branches each season. At flowering and prior to removing lateral buds, the numbers of spur, terminal and one-year lateral flower buds were recorded for each branch. In the first season in New Zealand, flowering and fruit set was only recorded on Control 6 treatments as all Control trees were the same up to this point. In season 4 in New Zealand, flowering was only recorded in treatments where return bloom floral bud densities had been less than fruiting bud densities in previous seasons. These treatments were the higher bud densities: ASE 5 and 6 in both regions, Control 4 and 6 in Hawke' Bay and Control 6 in Nelson. After natural fruit drop was complete, and before hand-thinning, the number of fruit set on each floral bud was recorded, including buds that failed to set fruit.

Counts of the numbers of flower buds on each branch were converted to spur + terminal (S+T) and total (S+T + lateral) floral bud density using numbers per cm⁻² BCA. Fruit set responses were investigated on both individual branches and individual buds. Fruit set density (number of fruit.cm⁻² BCA) and mean fruit set per floral bud were calculated for each branch. Fruit set patterns on individual buds were examined by converting the number of S+T buds in each of the seven classes of fruit set i.e. zero to six fruit per bud, to a proportion of the total floral bud number for that branch. Data from the three branches per tree were averaged to give estimated tree means and treatment means in each season.

Levene's test was used to test whether the variation in S&T floral bud density from tree to tree within a treatment was similar for different treatments at each site (Tested using Genstat v 17, VSN

International, U.K.). Regression analyses were conducted using procedures in Genstat v17 to test whether the relationships between floral bud density and the proportion of buds setting zero fruit, proportion setting two-or-more fruit, mean fruit set per bud, and fruit set density were similar among seasons for each site. Within a site, where regression analyses for some of the individual seasons did not differ, a single regression for these seasons was compared with that for the other seasons. The validity of this was judged by F tests and comparing overall adjusted R^2 values, which are a measure of how well the set of models for all years data from a site fit the actual values. Linear regressions were used unless adjusted R^2 values were appreciably improved by using power functions. Meteorological data were retrieved from the closest recording station (within 0.25 to 9km) to the site.

Results

Although no chemical thinners were applied to the experimental trees during this research, most blocks were situated within commercial orchards and some limited spray drift may have occurred in Hawke's Bay in seasons 2 and 3. In Nelson, less rain fell over the 21-day flowering period in season 2 (33mm) compared with seasons 1 and 3 (166mm and 145mm), and during the period 2 to 4 weeks after bloom, total radiation in season 2 (386 MJ.m^{-2}) was 24% greater than in seasons 1 and 3 (311 and 312 MJ.m^{-2}). The Tasmanian orchard was situated on a south-east facing slope and trees were planted on ridges to combat very wet winter soil conditions. Compared with other sites, these trees did not produce strong growth during this study, which appeared to be the consequence of wet soil during early season growth. In seasons 1 and 4, 170 and 195 mm of rain fell during October and November resulting in very wet soil during flowering and fruit set, compared with 76 mm in season 3. Seasons 1 and 4 also recorded single frost events of -1.2°C during flowering whereas season 3 did not. Frosts of -1 to -2°C were common between budbreak and flowering in Victoria, however only one occurred in season 1, while 3 to 5 occurred in each of the other seasons.

3.1 Flowering responses

Natural total floral bud densities in Control trees varied widely among sites and seasons, from about 4, to over 18 buds.cm^{-2} BCA (Table 2). In general, Control trees in Nelson produced the highest total floral bud densities and those in Tasmania and Queensland the lowest. The latter two regions showed large annual variation in total floral bud densities, while Nelson and Hawke's Bay were

consistently high among seasons. There was very little indication of any influence of the previous season's crop load on floral bud density in the subsequent season at any site. Spur and terminal (S+T) floral bud density in Control trees responded similarly to total floral bud density and was generally highest in Hawke's Bay and Nelson and lowest in Tasmania and Queensland. In contrast, floral bud density on one-year-old laterals varied widely among seasons irrespective of site. In some situations this bud type contributed as much as half of the total floral bud density, however, typically lateral buds comprised less than 30% of total floral bud density.

Table 2. The average natural density (number per cm² branch basal cross-sectional area (BCA)) of spur + terminal (S+T), one-year-old lateral (L) and total (Tot, S+T+L) floral buds on Control 'Gala' trees recorded at flowering in four seasons and five regions. These trees were unthinned until fruiting bud densities (number of fruiting buds per cm² BCA) were set by hand thinning after completion of natural fruit drop each season.

Fruiting bud density (number.cm ⁻² BCA)	Season 1			Season 2			Season 3			Season 4		
	S+T	L	Tot	S+T	L	Tot	S+T	L	Tot	S+T	L	Tot
Hawke's Bay												
2	-	-	-	14.0	2.9	16.9	13.6	0.8	14.4	-	-	-
4	-	-	-	13.2	2.1	15.3	12.5	0.6	13.2	12.3	1.4	13.7
6	8.6	0.7	9.3	11.3	2.1	13.4	11.5	0.5	12.0	10.7	1.3	12.0
Nelson												
2	-	-	-	11.7	3.8	15.6	10.8	1.8	12.5	-	-	-
4	-	-	-	13.3	4.5	17.9	14.2	2.7	16.9	-	-	-
6	8.4	9.6	18.0	12.5	5.7	18.3	13.9	3.4	17.3	10.7	2.6	13.3
Queensland												
3	7.5	0.8	8.3	9.4	6.4	15.7	8.4	3.5	11.9	6.1	1.1	7.2
4	6.6	0.9	7.5	8.2	7.9	16.1	9.4	2.5	11.9	5.7	0.5	6.2
5	5.5	0.7	6.2	8.4	6.3	14.8	9.0	2.3	11.3	4.1	0.4	4.6
Tasmania												
3	5.1	0.2	5.3	10.0	2.6	12.5	8.1	1.9	10.0	7.7	1.6	9.4
4	4.4	0.0	4.4	7.6	3.0	10.6	6.1	0.8	6.9	9.0	3.0	12.1
5	6.0	0.7	6.6	8.1	1.6	9.7	7.0	3.2	10.2	7.0	1.7	8.8
Victoria												
3	5.9	1.3	7.2	8.3	6.0	14.3	10.4	3.2	13.6	6.4	2.3	8.7
4	6.4	0.9	7.3	7.0	2.8	9.7	9.7	2.5	12.2	8.4	2.3	10.6
5	7.0	0.9	7.9	9.8	4.3	14.0	8.6	2.7	11.2	9.3	3.2	12.5

Table 3. The effect of a series of target fruiting bud densities (2 to 6 buds.cm⁻² branch basal cross-sectional area) set before bud break using artificial spur extinction, on actual density of spur + terminal (S+T), one-year-old lateral (L) and total (Tot, S+T+L) floral buds on 'Gala' trees at flowering in four seasons and five regions. At all sites apart from Victoria, flowers on one-year-old lateral buds were removed after counting.

Target fruiting bud density (number.cm ⁻² BCA)	Season 1			Season 2			Season 3			Season 4		
	S+T	L	Tot	S+T	L	Tot	S+T	L	Tot	S+T	L	Tot
Hawke's Bay												
2	2.3	0.0	2.3	2.9	6.3	9.2	2.5	3.8	6.3	-	-	-
3	3.0	0.0	3.0	3.5	8.0	11.5	3.8	5.1	8.9	-	-	-
4	3.9	0.0	3.9	4.5	6.5	11.0	4.4	2.8	7.2	-	-	-
5	4.5	0.0	4.5	5.1	4.4	9.5	5.5	2.5	8.0	4.5	3.1	7.6
6	4.6	0.0	4.6	5.5	5.4	10.9	5.8	1.8	7.6	5.1	1.9	7.0
Nelson												
2	2.8	-	-	2.0	7.4	9.5	2.9	8.7	11.5	-	-	-
3	3.6	-	-	3.9	7.9	11.9	3.9	9.2	13.1	-	-	-
4	3.8	-	-	4.6	7.5	12.1	3.9	7.2	11.1	-	-	-
5	4.5	-	-	5.8	7.5	13.3	5.2	5.2	10.4	5.3	9.4	14.7
6	5.9	-	-	6.3	7.9	14.2	6.0	6.4	12.4	6.5	6.8	13.3
Queensland												
3	3.2	0.0	3.2	3.6	-	-	2.9	1.6	4.5	2.2	0.5	2.7
4	3.5	0.0	3.5	4.0	-	-	3.8	2.3	6.1	1.4	0.2	1.6
5	4.4	0.0	4.4	5.4	-	-	4.9	1.7	6.6	2.0	0.1	2.1
Tasmania												
3	2.5	0.0	2.5	3.7	2.9	6.6	3.3	4.4	7.7	3.6	0.5	4.0
4	3.0	0.1	3.1	4.4	1.6	6.0	3.6	2.1	5.7	4.4	0.3	4.6
5	3.0	0.0	3.0	5.1	1.2	6.3	4.8	4.4	9.3	6.0	0.2	6.1
Victoria												
3	3.1	1.6	4.7	3.5	3.5	7.0	3.2	4.8	8.0	2.8	5.1	7.9
4	4.1	1.3	5.4	4.4	4.2	8.7	4.5	4.3	8.7	3.0	3.1	6.2
5	4.1	0.8	4.9	5.4	3.7	9.2	5.2	5.9	11.1	4.1	3.4	7.5

Within each site and season, in almost all situations, removal of dormant buds in winter using ASE, reduced the total density of buds that flowered in spring compared with the controls (compare Tables 2 and 3). In ASE treatments, at sites where lateral buds were removed at flowering (all sites apart from Victoria) floral load was reduced to the S+T floral bud density i.e. between 1.4 and 6.5 buds.cm⁻² BCA (Table 3). ASE treatments set to carry buds at the commercial fruiting bud densities of 4 (Australia) and 5 (New Zealand) buds.cm⁻² BCA generally carried floral bud densities of within 0.8

buds.cm⁻² BCA of this, i.e. using ASE, ≥80% of buds set in late dormancy flowered in spring. Lower floral bud densities in seasons 1 in Tasmania and 4 in Queensland were associated with lower floral bud densities in Control trees as well.

Tests for differences in the variability of S&T floral bud density among treatments at each site were significant in 12 out of 18 cases (p-values ranged from <0.001 to 0.973). Investigation of these data showed that standard deviations of individual tree floral bud densities around the treatment mean floral bud density for each site x season, increased with increasing treatment mean floral bud density (Figure 1). Lower floral bud densities in ASE treatments resulted in standard deviations that were almost always less than 1.5, whereas standard deviations of most of the Controls were greater than 1.5 and reached 4 in some cases. Within a site and season, standard deviations of ASE treatments were generally half that of the Controls.

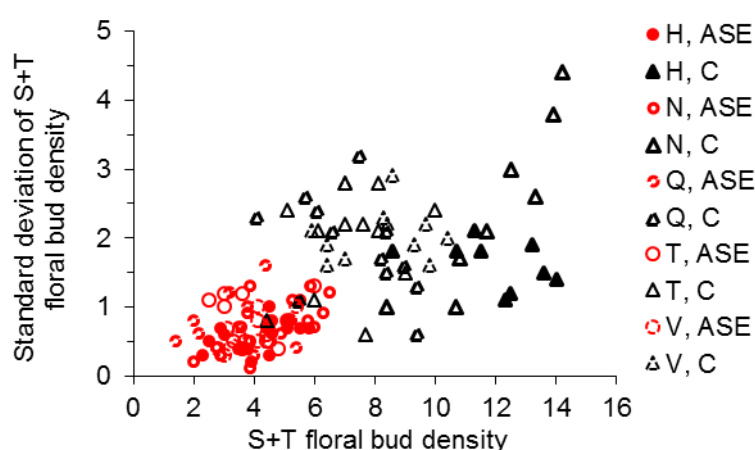


Figure 1. The effect of spur + terminal (S+T) floral bud density (number of buds.cm⁻² branch basal cross-sectional area, FBD) on the standard deviation of S+T FBD among ‘Gala’ trees managed using either artificial spur extinction (ASE) or post fruit drop hand thinning (C, control) over five regions (Hawke’s Bay (H), Nelson (N), Queensland (Q), Tasmania (T) and Victoria (V)) and four seasons (not distinguished in plot). Each S+T FBD value is the mean of 5 trees, or, in Nelson, 4 trees within a season.

3.2 Fruit set within individual spurs

Preliminary data investigating the proportions of floral buds setting from zero to six fruit per bud in the first season in Hawke’s Bay, Nelson and Tasmania, and the first two seasons in Victoria and Queensland have been presented by Breen et al. (2014). In Control trees, at most sites the inclusion

of subsequent seasons of data did not alter the proportions (about 10 to 30%) of floral S+T floral buds that failed to set fruit (Figure 2, triangles). In Queensland, while in the first two seasons 50-60% of floral S+T buds failed to set fruit, this proportion was lower in the third (19%) and fourth (44%) seasons. Low seed numbers in fruit at harvest in season 2 suggested that pollination was limiting in this block and consequently in subsequent seasons, flower bouquets were placed through the block. In Control trees in Hawke's Bay and Victoria, seasonal differences in the proportions of buds setting zero fruit varied by less than 10 units. In Nelson and Tasmania, in most seasons the proportion of buds setting zero fruit was about 10%, however in Tasmania in season 4 this proportion increased to 22% and in Nelson in season 3 to 27%. In Control trees, among seasons within every site, differences in the proportion of buds setting zero fruit, were independent of S+T and Total floral bud densities.

As in the preliminary data presented by Breen et al. (2014), subsequent results showed that in Control trees the relative proportion of buds setting two-or-more fruit declined as the number of fruit set per bud increased (not shown). The proportion of buds setting three or more fruit never exceeded 25% of the total number of floral buds (not shown). The only exception to this was in Tasmania in season 3, where unusually high proportions of buds set 3 and 4 fruit per bud (~25% in each case). In Control trees, the proportion of buds setting two-or-more fruit varied widely among sites and was not usually associated with differences in floral bud density among seasons (Figure 3, triangles).

In Control trees, in almost all cases, buds setting one fruit formed the greatest proportion (30-50%) of floral buds, and within any site, this proportion varied by less than 12% among seasons (compare Figures 2 and 3)(Breen et al., 2014). However, Queensland and Tasmania provided exceptions. In Queensland, season 3 followed this trend, but in all other seasons the proportion setting zero fruit was twice to three times greater than those setting one fruit. In Tasmania, season 3 was the exception with only 10% of buds setting one fruit compared with ~30% in other seasons.

Reducing floral bud density through application of the series of ASE treatments reduced the proportion of floral buds setting zero fruit (Figure 2, circles) and increased the proportion setting two-or-more fruit (Figure 3, circles) without exception, across all sites and seasons, and irrespective of natural floral bud density occurring in Control trees. In many cases, ASE treatments also reduced the proportion of floral buds setting one fruit compared with the Controls (not shown).

Within sites, linear (Hawke's Bay, Nelson and Tasmania) and power (Queensland and Victoria) functions described 6 – 99% of the variation in the proportion of buds setting zero (median 75%), or two-or-more fruit (median 83%) within seasons. R^2 values for Victoria in season two were low (18% and 6% respectively). Median values being much greater than the mid-point of the range confirmed that low R^2 values achieved in some seasons were atypical. Within each site, the responses of the proportion of floral buds setting zero or two-or-more fruit to floral bud density were similar in some seasons. Grouping the similar seasons and describing them using one response curve (Figures 2 and 3) only altered 'overall adjusted' R^2 values by -5.6 to +2.1 % and significantly separated them from the remaining season ($P=0.047$ to <0.001). In Victoria all seasons had similar fruit set responses. Within a site, differences between season groups were generally limited to differences in y intercept value. However in season 3 in Queensland and seasons 2 and 3 in Hawke's Bay, there was also a lower response (i.e. the slope was not as steep) compared with other seasons within the site.

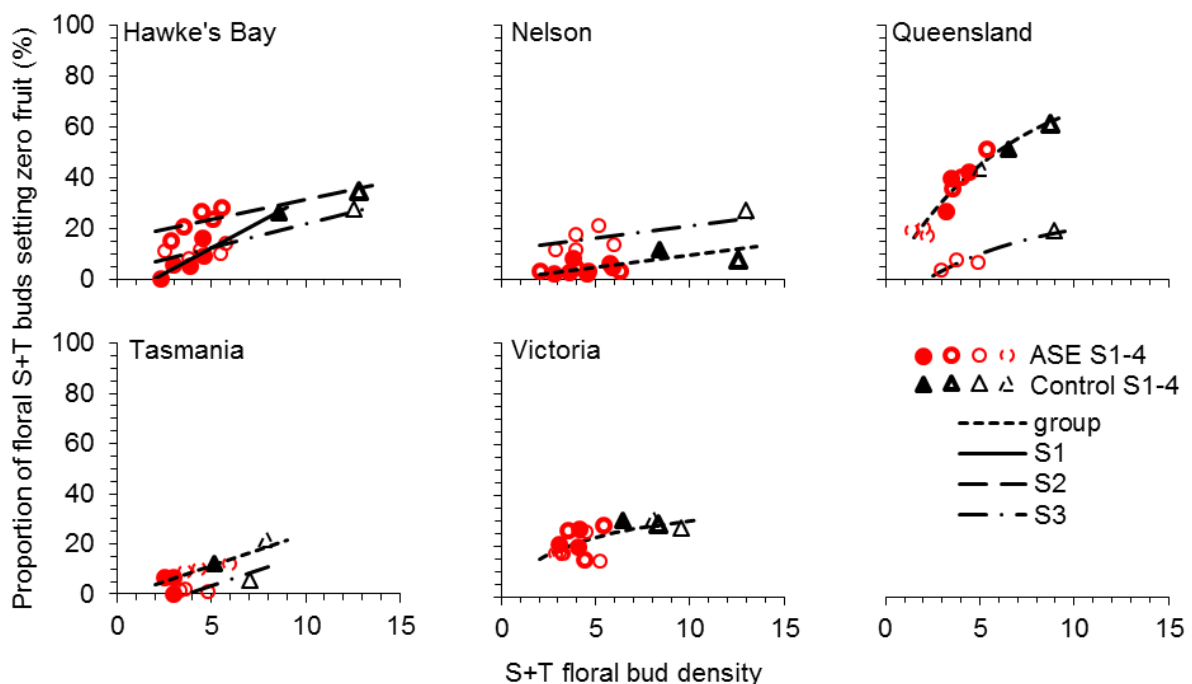


Figure 2. The effect of Spur + Terminal (S+T) floral bud density (number of buds.cm⁻² branch basal cross-sectional area, FBD) on the proportion of floral S+T buds setting zero fruit in 'Gala' trees in 5 regions over 4 seasons (S1 to S4). FBD was unmodified in Control trees or set to a series of S+T bud densities (with lateral buds removed apart from in Victoria) using artificial spur extinction (ASE). A

common line of fit is presented for all seasons (group, G) apart from where individual season lines (S1 to S3) differed ($P \leq 0.05$) from the common line (S4 did not differ from the group in any season or region).

Figure 3. The effect of Spur + Terminal (S+T) floral bud density (number of buds.cm⁻² branch basal cross-sectional area, FBD) on the proportion of floral S+T buds setting two-or-more fruit in 'Gala' trees in 5 regions over 4 seasons (S1 to S4). FBD was unmodified in Control trees or set to a series of S+T bud densities (with lateral buds removed apart from in Victoria) using artificial spur extinction (ASE). A common line of fit is presented for all seasons (group, G) apart from where individual season lines (S1 to S3) differed ($P \leq 0.05$) from the common line (S4 did not differ from the group in any season or region).

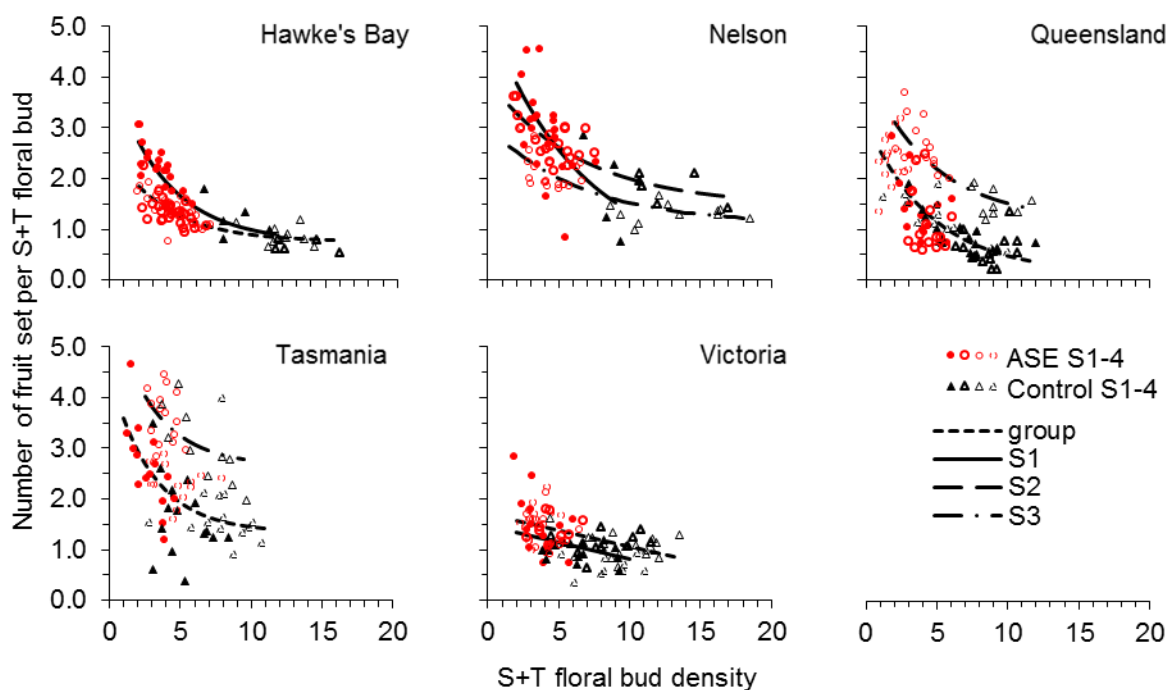


Figure 4. The effect of Spur + Terminal (S+T) floral bud density (number of buds.cm⁻² branch basal cross-sectional area, FBD) on mean number of fruit set per floral S+T bud in 'Gala' trees in 5 regions over 4 seasons (S1 to S4). FBD was unmodified in Control trees or set to a series of S+T bud densities (with lateral buds removed apart from in Victoria) using artificial spur extinction (ASE). A common line of fit is presented for all seasons (group, G) apart from where individual season lines (S1

to S3) differed ($P \leq 0.05$) from the common line (S4 did not differ from the group in any season or region).

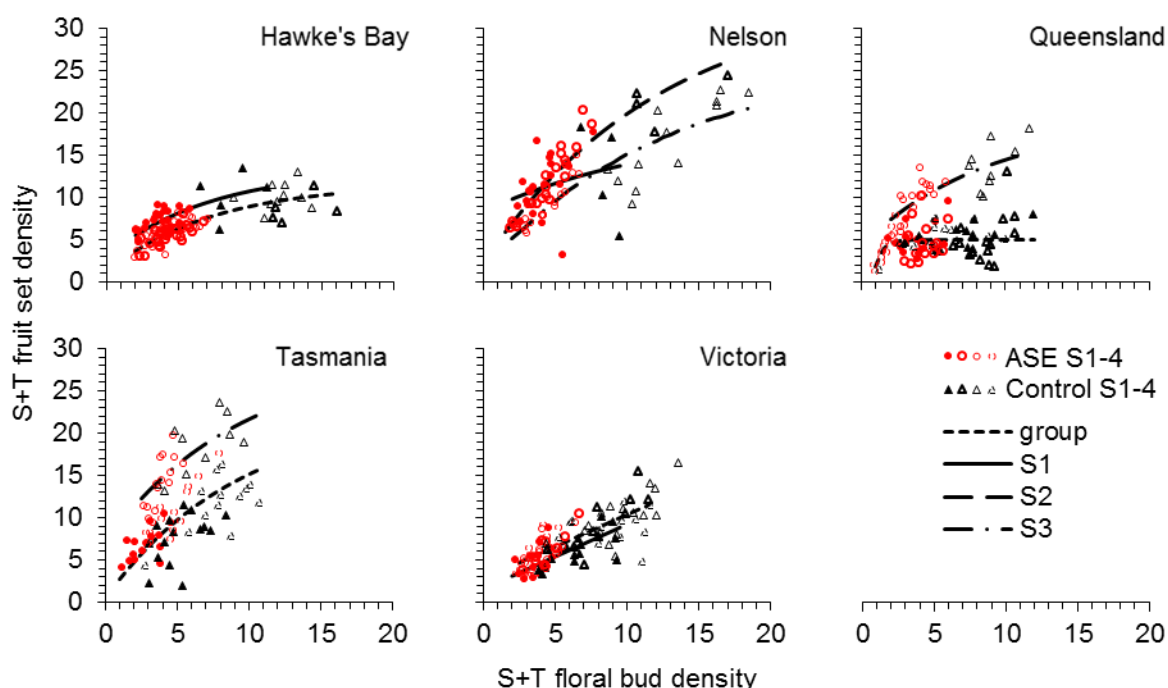


Figure 5. The effect of Spur + Terminal (S+T) floral bud density (number of buds.cm⁻² branch basal cross-sectional area (BCA), FBD) on the number of fruit set on S+T buds per cm² BCA (fruit set density) in 'Gala' trees in 5 regions over 4 seasons (S1 to S4). FBD was unmodified in Control trees or set to a series of S+T bud densities (with lateral buds removed apart from in Victoria) in artificial spur extinction (ASE). A common line of fit is presented for all seasons (group, G) apart from where individual season lines (S1 to S3) differed ($P \leq 0.05$) from the common line (S4 did not differ from the group in any season or region).

3.3 Mean fruit set per bud

The mean number of fruit set per S+T floral bud (mean fruit set) declined from between 1.5 and 4.5 fruit per bud at a S+T floral bud density of 2, to between 0.5 and 2.5 fruit per bud at a floral bud density of 12 across all treatments, in all sites and seasons (Figure 4). However, each site expressed a distinctive set of response curves. Power functions at most sites, and linear functions in Victoria, described 10 to 72% (median 51%) of the variability in the data within seasons.

Within most sites, some seasons showed similar fruit set responses. Regression analysis showed that these seasons produced response curves that differed from other seasons, and grouping similar

response curves did not reduce overall adjusted R^2 values. In Hawke's Bay, the Y asymptote x rate value for season 1 was greater ($P<0.001$) than a combined response for seasons 2 and 3, showing that greater numbers of fruit set per bud at low floral bud densities in season 1 (Figure 4). In Nelson, season 1 which showed much greater variability in mean fruit set at natural S+T floral bud densities, displayed a greater Y asymptote x rate value ($P=0.035$) than seasons 2 and 3, producing a much steeper response of mean fruit set to floral bud density. The Y asymptote value also varied between seasons 2 and 3 ($P=0.05$), season 3 setting ~0.6 fewer fruit per bud than season 2. Queensland and Tasmania produced similar responses to each other. At these sites in season 3 mean fruit set was 1.1 (Queensland) and 1.3 (Tasmania) fruit per bud greater ($P<0.001$) than in other seasons combined. Differences between seasons 1 and others in Victoria were significant ($P<0.001$), but small (0.2 fruit per bud).

3.4 Fruit set density

All sites and seasons showed a positive relationship between S+T floral bud density and the number of fruit set per cm^2 branch basal cross-section area (fruit set density, Figure 5). At low floral bud densities fruit set density was generally in the range 2 – 5 fruit per cm^2 . At high floral bud densities, fruit set density varied widely among sites and seasons but did not exceed 25 fruit. cm^{-2} . In general, in Hawke's Bay, Queensland and Victoria fruit set density was less responsive to floral bud density (showed flatter response curves) than Nelson and Tasmania. Most sites showed a diminishing marginal response of fruit set density to S+T floral bud density for each season, best described by power functions. In Victoria, linear relationships provided better estimates of the seasonal responses. At all sites, 0.2 to 89% (median 61%) of the variation in seasonal fruit set density could be accounted for by these response curves.

Within sites, seasons in which mean fruit set response differed from others (Figure 4) also showed different fruit set density responses to floral bud density (Figure 5). As with the relationships between mean fruit set and floral bud density, the fruit set density : floral bud density relationships showed that within sites most seasons could be described by a single curve with very little change in overall adjusted R^2 value ($<5\%$). When seasons 2 and 3 in Hawke's Bay were plotted as a single response curve, they were shown to set 1.8 fewer fruit per cm^2 than season 1 (Y asymptote difference $P<0.001$) (Figure 5). Fruit set density in season 1 in Nelson was much less responsive to floral bud density than seasons 2 and 3 ($P=0.01$). Season 2 had a greater Y asymptote x rate value ($P=0.035$) than

season 3, resulting in small differences in fruit set density at low floral bud densities (~2 fruit at 2 buds.cm⁻²) and large differences at high floral bud densities (~6 fruit at 16 buds.cm⁻²). The response curve for season 3 in Queensland was completely different (all parameters differed, $P < 0.001$) from a combined curve for the other three seasons. At low floral bud densities season 3 set ~3 fruit per cm² more fruit than the other seasons combined while at high floral bud densities this difference was 10 fruit per cm². In Tasmania, season 3 differed from combined seasons 1 and 4, setting 6.6 more fruit per cm² (Y asymptote difference $P < 0.001$). Linear regression in Victoria showed a small but significant (0.4 fruit per cm², Y intercept difference $P = 0.003$) decrease in fruit set per cm² in season 1 compared with the other seasons combined.

Discussion

Heavy flowering is often regarded as critical to economic success in commercial apple production. Breeding of apple scion genotypes and use of dwarfing rootstocks has led to a situation where cultivars in commercial production such as 'Gala' regularly produce more than ten times more flowers per tree than are required for a commercial crop. Consequently, growers must greatly reduce flower and fruit numbers as early in the season as possible to optimise yield and fruit quality. To ensure commercial yields and fruit quality, early reduction in flower or fruit numbers must produce a reliable and predictable outcome. Current commercial reliance on chemical thinning does not achieve this.

4.1 Flowering responses

In this study, some of the challenges facing commercial producers were clearly shown in the results of annual flowering on Control trees. Floral bud density varied widely among seasons within a site, often by two or three times the lowest floral bud density for the site, even among trees with no indication of a biennial flowering pattern (Table 2). In Hawke's Bay, Nelson and Queensland in some seasons four times more flower buds were present than were required to produce a commercial crop even if each bud only held a single fruit (4 and 5 buds.cm⁻² BCA in Australia and New Zealand respectively). In Nelson and Queensland, in some seasons half of the total floral bud density was comprised of one-year-old lateral buds which produce smaller fruit (Volz et al., 1994) and are typically removed in commercial production. Even in seasons where the lowest floral bud densities were recorded, these would have been sufficient to produce a commercial crop on S+T buds alone if each cluster set one fruit. Natural flowering intensity also varied among trees within site and season, by at least 1.5 and

up to 4 times the season x site mean (Figure 1). It is very difficult for commercial growers to reduce these flower (and developing fruit) numbers cogently, while both seasonal climatic differences and widely differing floral bud densities among seasons and trees affect the fruit set response.

Removal of buds prior to bud break using ASE greatly improved the predictability in flowering response. Floral bud densities were generally reduced appreciably, and more than 70% of the buds selected flowered (Table 3). Where ASE was set at commercial fruiting bud densities (4 in Australia and 5 in New Zealand), usually more than 80% of these buds flowered. Variation in floral bud density among trees, sites and seasons was generally halved compared with the control (Figure 1). In seasons 1 in Tasmania and 4 in Queensland, lower floral bud densities were observed over all treatments and target floral bud densities were not achieved (Tables 1 and 2). This appeared to be the result of poor floral bud development, possibly induced because management was unable to respond sufficiently to environmental conditions. In Queensland this was a district-wide phenomenon and may have been the result of drought and irrigation limitations during floral bud development the previous summer. In Tasmania waterlogging may have inhibited floral bud development in the previous season. However, in some situations in ASE trees in the first season, the probability of a bud selected at bud thinning being floral is lower than in subsequent seasons. At this stage the proportion of floral buds in the canopy is lower as it contains a high proportion of vegetative buds, particularly if there was a heavy crop the previous season. In subsequent seasons there are fewer vegetative buds as ASE induces the bourse-over bourse phenomenon (reiterative flowering on sequential terminal buds) (Lauri et al., 2004).

4.2 Fruit set responses

The maximum fruit set density of ~25 fruit per cm² BCA (Figure 5) observed in Nelson and Tasmania may represent a genotype maximum response to carbon resource availability for fruit set on a branch basis. Reduced fruit set density achieved in other seasons and sites may illustrate the effects of climate and environment on reduced carbon availability, but also includes the effects of factors such as frost damage and pollination success. Although there were clear responses of fruit set to floral bud density over the whole range of floral densities recorded, at densities greater than 8-10 buds per cm² BCA, such as those measured on Control trees, relationships between floral bud density and fruit set were weak. At these high floral bud densities, the proportion of buds setting zero fruit per bud (Figure 2) or two-or-more fruit per bud (Figure 3), as well as mean fruit number per bud (Figure 4) and fruit set

density (Figure 5) were unaffected by floral bud density, and this was evident among sites and seasons. The responses of number of fruit set per bud and fruit set density to floral bud density were also usually more variable among trees at floral bud densities above 8-10 buds per cm² BCA (Figures 4 and 5).

Reducing floral bud density using ASE achieved more predictable responses to fruit set. Mean fruit number per bud increased strongly when floral bud densities declined below 6 buds.cm⁻² BCA. This was the result of smaller proportions of buds setting zero fruit and increased proportions setting two-or-more fruit. This result may be caused by reduced competition among developing fruit in the period 2 - 4 WAB when carbon supply is thought to be limited (Lakso et al., 1999; Lakso et al., 2006a).

However, research we have recently conducted (Breen et al., 2015; Breen et al., in press) has provided an alternative hypothesis that reduced bud density in ASE trees improves irradiance of the remaining fruit-bearing buds which likely increases net carbon assimilation and improves the carbon supply per developing fruit in the period 2 – 4 WAB. This hypothesis is supported by modelling of tree structure and light interception in Centrifugally Trained trees (Willaume et al., 2004; Stephan et al., 2008).

Commercial apple trees are generally thinned to a single fruit per bud to achieve optimum fruit size and quality. To increase yield, two fruit per bud are often retained in parts of the canopy having high light intensity and on fruit buds with strong growth. To maintain yield, as floral bud density declines, the requirement for remaining floral buds to set at least one fruit increases, because commercial yields and quality cannot be retained using more than 2 fruit per cluster. At all sites and in every season, reducing floral bud density using ASE strongly reduced the proportion of floral buds setting zero fruit and strongly increased the proportion setting two-or-more fruit. When ASE was set at commercial bud densities (4 to 5 buds.cm⁻² BCA), in most seasons 80-90 % of floral buds set at least one fruit and in all sites and seasons more than 30% of floral buds set two-or-more fruit.

Consequently, in all seasons apart from season 4 in Queensland, when final crop loads were set, thinning to two fruit per bud on some of the buds that set two-or-more fruit, compensated for those setting zero fruit.

Terminal buds of short to medium length (2.5-30 cm) shoots produce bourses and bourse shoots with greater leaf area than those of spur buds (Tustin et al., 2011b). The proportion of short to medium length shoots is increased in ASE-treated trees compared with unmodified trees (van Hooijdonk et al.,

2010; Tustin et al., 2011a), and irradiance of these shoots is also thought to be greater in ASE treatments. Consequently, thinning to two fruit per bud on 10 - 20% of buds in trees where bud densities have been reduced using ASE is unlikely to compromise fruit size and quality in 'Gala'.

4.3 Modelling of fruit set responses

Tustin et al. (2012) and Breen et al. (2014) have suggested that models may be developed to predict fruit set responses among seasons on trees thinned using ASE. In results presented here, fruit set responses within each site were similar in most seasons. In seasons where fruit set responses differed, this could be explained by variation in environmental conditions such as weather and orchard management. In Hawke's Bay, in seasons 2 and 3 some drift of thinning sprays may have occurred during application to neighbouring trees. This may explain the similarity in fruit set between seasons 2 and 3 which differed from season 1. The lower proportion of buds setting two-or-more fruit, and reduced mean fruit set and fruit set density in seasons 2 and 3, implies that the thinner used had greater effect on thinning buds holding two-or-more fruit. Consequently a fruit set model for these trees in the absence of thinning sprays (season 1) and using ASE to manage floral bud load, predicts that at a commercial floral bud density of 5 buds.cm⁻², 12% (95% confidence interval of +/- 3%) of floral buds will set zero fruit, 60% (+/- 6%) will set two-or-more fruit and therefore 28% (+/- 4%) will set a single fruit per bud.

In Nelson, seasons 1 and 3 had similar climatic conditions but fruit set responses appeared to differ. At this site, this may have been the result of greater variability in the responses of mean fruit set and fruit set density to floral bud density in season 1, which reduced the accuracy of predicting the season 1 responses. Greatly reduced rainfall and 24% greater total incident radiation during flowering and fruit set in season 2 was associated with increased fruit set compared with season 3. In season 2, at a commercial floral bud density of 5 buds.cm⁻² BCA, 13% fewer buds set zero fruit, 18% more buds set two-or-more fruit, mean fruit set per bud increased by 0.8 units and fruit set density increased by 3.3% compared with season 3. In Tasmania, high rainfall during flowering and fruit set in seasons 1 and 4 was associated with reduced fruit set compared with season 3 which had low rainfall. Rainfall may have reduced incident radiation and carbohydrate availability during the period 2 – 4 weeks after bloom when carbohydrate limitation reduces fruit set. However, in Nelson and Tasmania, reduced pollinator activity through rainfall, and periods of reduced incident radiation (Vicens and Bosch, 2000), and waterlogging in Tasmania may have induced fruit abscission. Single frost events of ~1.2°C

during flowering in Tasmania in seasons 1 and 4 may also have contributed to this fruit set response. However, although cultivars show differing sensitivities to frost damage (Lipa et al., 2008) these temperatures are probably not low enough to have caused significant damage (Proebsting and Mills, 1978).

In Queensland, in seasons 1 and 2, exceptionally low fruit set at high floral bud densities, low density of polliniser trees, and an orchard completely enclosed with hail netting suggested that pollination was a limiting factor in fruit set. In season 2, low seed counts in harvested fruit supported this view.

Flower bouquets of another apple cultivar were placed through the orchard during flowering in seasons 3 and 4. In season 3, this significantly improved fruit set and resulted in fruit set responses similar to other sites, and in season 4, a lower proportion of buds set zero fruit and a greater proportion set 2 or more fruit. Target floral bud densities were not achieved in seasons 4 in Queensland and 1 in Tasmania. However, season 4 in Queensland was the only season where too few buds set fruit to achieve target crop loads of 4 and 5 fruit.cm⁻² BCA in ASE trees. In this season, a combination of poor floral bud development and inadequate pollination may have resulted in a low proportion of buds setting one fruit, while the proportion of buds setting two-or-more fruit was insufficient to compensate for those setting zero fruit. Slightly higher S+T floral bud densities in Control trees suggest that ASE treatments would have held sufficient floral buds if the ASE process had been imposed closer to budbreak, so that floral buds could have been more easily distinguished. On 'Gala', trees managed using ASE produce more buds than are required every season because previously extinct sites regenerate, and bourse structures may produce two shoots.

Fruit set responses in Victoria were very similar in all four seasons and, although statistically season 1 had a lower mean fruit set and fruit set density than the other seasons, differences were small (0.2 fruit per bud and 1.2 fruit.cm⁻² BCA). Within this site the difference between these seasonal fruit set responses could not be explained by the number of frost events occurring between budbreak and flowering. It is possible that the generally lower fruit set in Victoria compared with other sites may be explained by the generally higher incidence of frost between budbreak and flowering at this site. However, the presence of lateral flower buds at this site may also have reduced overall fruit set on S+T buds through increased competition for limited carbon resources.

Of particular interest are the results from Nelson and Queensland. Within these sites, seasonal response curves of fruit set density (Figure 5) and of the proportion of floral buds setting zero fruit

(Queensland) (Figure 3) in relation to floral bud density were not parallel. Divergence of these curves with increasing floral bud density suggested that where ASE reduced floral bud densities, the fruit set response among seasons was moderated. In treatments where floral bud density is reduced using ASE, fruit set is thought to be increased through increased carbohydrate availability over the 2 – 4 WAB period (Breen et al., 2015; Breen et al., in press), during which time there is high demand for limited carbohydrate resources. If the fruit set responses to floral bud density were entirely the effect of the number of competing sinks, differences in incident radiation among seasons in the 2 – 4 WAB period, such as observed in Nelson, could be expected to produce parallel response curves. The divergence of these response curves in Nelson could be because the improved light environment of each fruiting bud in ASE treatments may moderate the fruit set response through improved carbohydrate availability. This could result in fruit set in ASE trees being less affected by reduced incident radiation than Control trees. In Queensland, where pollination was limited through insufficient pollen availability, reduced floral bud density in ASE trees may have enabled more thorough pollination of remaining flowers resulting in improved fruit set. Commercially these responses are important as they show that compared with Control trees, where floral bud density has been reduced using ASE, fruit set responses are more reliable among seasons that vary in environmental aspects such as weather and pollination.

Conclusions

Natural flowering and fruit set on unmodified (Control) trees differed widely among trees, sites and seasons even in trees that were not irregular bearers. Even in seasons where floral bud development had been impaired, the number of S+T flowering buds was always sufficient to produce a commercial crop if each bud set one fruit. At most sites, there were seasons when Control trees produced four times more floral buds than required to carry a commercial crop load at one fruit per bud. Natural fruit set in Control trees, especially at high floral bud densities was highly variable, largely unresponsive to floral bud density and often highly responsive to differences in weather, orchard conditions and pollination factors. These responses are often observed in commercial apple production and are among the reasons why commercial apple producers generally regard heavy flowering and fruit set to be important in securing high final yields.

Delays in reducing fruit numbers may negatively affect potential fruit size and quality and induce irregular bearing. For these reasons, growers seek to reduce natural crop load early. However, current commercial practice using chemical thinning does not produce reliable and predictable outcomes. In this research, when Control trees produced naturally lower floral bud densities there was no improvement in predictability of fruit set responses. However, systematic reduction of bud density using ASE did produce predictable flowering and fruit set responses. When ASE was set at commercial bud densities variability in floral bud density among trees, sites and seasons was greatly reduced and floral bud density generally declined to within 20% of target floral bud density. When ASE was applied, reliability in the relationship between fruit set responses and floral bud density allowed models to be fitted for each site which described relationships between fruit set and floral bud density. At all sites apart from Victoria, these models generally explained >50 - 94% of the variability in the data and described altered fruit set in response to changes in climate and environment. In Victoria, fruit set was generally less responsive to floral bud density. Retaining lateral flower buds at the Victoria site may have contributed to this attribute.

Consequently, use of ASE in 'Gala' provided a method of very early crop load adjustment which produced reliable and predictable outcomes to fruit set over a number of sites and seasons.

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Chapter 3. Method of manipulating floral bud density affects fruit set responses in apple

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Abstract

Apple trees (*Malus x domestica* Borkh.) usually produce abundant flowers and fruit, and numbers must be reduced to achieve commercial targets of fruit size and quality. Profuse flowering and fruiting also creates high demand for a limited source of carbohydrates, which affects fruit set and development. Artificial spur extinction (ASE) uses hand-thinning of whole buds in late dormancy to reach targeted floral bud densities on every limb. ASE removes floral and vegetative sinks and potential leaf area. Consequently, ASE provides a useful tool to improve understanding of the physiological processes affecting fruit set. We investigated flowering, fruit set and yield of 'Royal Gala' trees treated with three thinning methods; ASE, flower cluster thinning (FCT) at pink bloom, and fruit thinning after final fruit set (Control). ASE and FCT were imposed at 2, 4 or 6 buds or flower clusters per cm² branch cross-sectional area (BCA). Final fruit thinning on all three thinning methods was imposed at two fruit per bud on 2 buds.cm⁻² BCA and a single fruit per bud on 4 and 6 buds.cm⁻² BCA. Consequently, although the density of fruit-bearing buds differed between 2 and 4 buds.cm⁻² BCA, fruit density did not. Compared with the Control, removal of floral sinks before flowering in ASE and FCT increased fruit set within flower clusters by more than 50% and increased harvest mean fruit weight by 18–32 g.fruit⁻¹. Compared with FCT, ASE resulted in marginally lower fruit set but did not

alter components of yield and had little effect on fruit quality. Results imply that manipulation of floral bud density may have beneficially altered carbon availability within floral spurs during early-season development. In FCT, reducing the density of flower clusters may have increased carbon availability to remaining fruit buds, improving their fruit set and development. In ASE, removal and uniform spatial distribution of buds may have improved irradiance of fruiting spurs, thereby increasing photosynthate availability to developing fruit on that spur, improving fruit set and development compared with the Control, achieving results similar to FCT. Despite large differences in natural fruit set brought about by normal seasonal differences in environment, ASE produced precise fruit set outcomes. This contrasts starkly with current commercial thinning practices that rely heavily on chemical thinning, and supports the use of ASE as a precision alternative to existing commercial crop load management.

Keywords

Flowering; thinning; artificial spur extinction; yield; crop load; carbohydrate

Introduction

The prolific production of flowers is an adaptive strategy observed in many plants in order to maximise production of mature fruit and seeds (Estornell et al., 2013; Celton et al., 2014). In crops such as apple, most commercial scion genotypes grown on dwarfing rootstocks produce many more flowers than the tree can support to fruit maturity, resulting in flower and fruit abscission (Tromp and Wertheim, 2005; Tustin et al., 2011a; Breen et al., 2014). In general, even after this natural abscission, large fruit numbers result in small fruit size and poor quality, and may lead to reduced cold hardiness, limb breakage and inhibition of floral initiation causing biennial bearing. (Dennis, 2000; Tromp and Wertheim, 2005; Estornell et al., 2013; Celton et al., 2014).

By the early 20th century, fruit thinning in apple orchards was an established commercial practice, although for many growers not standard practice (Gourley, 1922). It was not until the development of chemical thinning that the practice found greater use and it is now considered routine in commercial orchards (Dennis, 2000; Tromp and Wertheim, 2005; Schroder et al., 2013; Celton et al., 2014).

Despite 70 years of research, the mode of action of thinning chemicals, particularly plant bioregulators, remains unclear (Dennis, 2000; Schroder et al., 2013). Greater knowledge of the

mechanisms regulating fruit set and abscission is needed to improve commercial thinning practices (Lakso, 2011; Estornell et al., 2013).

In apples, fruit set and development relies on carbohydrates and nitrogenous compounds sourced from remobilised reserves and current photosynthesis (Titus and Kang, 1982; Oliveira and Priestley, 1988; Tromp, 2005). The contribution of reserves to early-season growth is difficult to assess as not all chemically extractable reserves are physiologically “available” (Lakso et al., 1999). Budbreak consumes a considerable portion of reserves, however after bloom, reserves are thought to play only a minor role in carbohydrate supply (Priestley, 1970; Hansen and Grauslund, 1973; Oliveira and Priestley, 1988; Lakso et al., 2006b).

At full bloom, primary spur leaves constitute most of the leaf area of the spur. Their presence is critical to fruit set and a decrease in primary spur leaf area reduces fruit set (Ferree and Palmer, 1982; Proctor and Palmer, 1991; Lauri et al., 1996). Primary spur leaves on both fruiting and non-fruiting spurs are able to supply photosynthates to developing flowers from pink bud and are the major source of carbohydrates for fruit development until 3–5 weeks after bloom (WAB) (Ferree and Palmer, 1982; Tustin et al., 1992; Corelli Grappadelli et al., 1994). In early spring, developing extension and bourse shoots are stronger carbon sinks than fruit and their presence may reduce fruit set (Quinlan and Preston, 1971; Ferree and Palmer, 1982). At 2–3 WAB, bourse shoots become net carbon exporters to fruit on the same spur but may also supply carbohydrate to fruit on neighbouring spurs. Their presence during this period is essential to increasing final fruit set (Quinlan and Preston, 1971; Ferree and Palmer, 1982; Tustin and Lai, 1990). Early termination of bourse shoot growth improves assimilate partitioning to developing fruit (Tustin et al., 1992).

Cell division is a more energy-expensive process than cell expansion or carbohydrate accumulation (Lakso and Denning, 1996). In apple fruit, cell division lasts for 3–6 weeks after bloom and total respiration per fruit increases up to a peak when fruit reach 15–20mm diameter (Blanpied and Wilde, 1968; Bepete and Lakso, 1997; Tromp and Wertheim, 2005). During this time, developing shoots appear to have priority for limited carbohydrate supply over the large numbers of fruit with exponential growth rates (Quinlan and Preston, 1971; Lakso et al., 1999; Lakso et al., 2001). Modelling of carbohydrate supply and demand curves for apple suggests that carbohydrate demand from fruit is likely to exceed supply in the period 2-4 weeks after full bloom and this deficit reduces fruit growth rate (Lakso et al., 1998; Lakso et al., 1999; Lakso, 2011). The abscission process is induced in the

slowest growing fruit when their growth rates decline below cultivar-specific critical levels (between 50 and 70%) compared with the fastest growing fruit (Greene et al., 2005; Lakso et al., 2006a).

Correlatively driven abscission (CDA) theory regards basipolar auxin flow as the dominant factor in determining fruitlet abscission (Bangerth, 2000). Autoinhibition of auxin (and possibly cytokinin) synthesis in the slowest developing fruit leads to critically low auxin concentration in their abscission zones, preventing continued ethylene inhibition thereby initiating abscission. The greater the fruit number within the cluster, the greater the autoinhibition in the slowest developing fruit and the greater the chance that it will abscise (Bangerth, 2000).

Much of the discussion in the literature concentrates on whether the primary signal for fruitlet abscission is through hormonally regulated processes, such as CDA, or by carbohydrate (or nutritional) status. It is difficult to explain fruit abscission solely in the context of either CDA or carbohydrate source-sink competition. It is also likely that the vast number of factors known to affect abscission interact through a common or integrated path, which leads to abscission (Bangerth, 2004; Lakso, 2011; Schroder et al., 2013). A recently proposed model links the CDA and carbohydrate competition models through sugar signalling (Botton et al., 2011; Eccher et al., 2014). Besides their importance for growth, sugars are thought to act as signalling molecules. In this model, Botton, Eccher and colleagues postulate that strong competition for assimilates among shoot and fruit sinks is “perceived” primarily by the cortex of the developing fruit as sugar (sucrose and trehalose) shortage stress. Persistent stress signalling stimulates biosynthesis of abscisic acid and ethylene, expression of key genes and signalling of isoprene and reactive oxygen species, all of which transmit signals to the seed. On reception of these signals by the seed, embryogenesis is blocked and seed abortion occurs, resulting in critically low polar auxin transport and abscission zone activation (Botton et al., 2011; Eccher et al., 2013; Eccher et al., 2014).

Morphological differences among genotypes also affect flowering and fruit set. Some apple genotypes such as ‘Rome Beauty’ and ‘Granny Smith’ exhibit a high degree of spur extinction. In this natural process, potential bourse shoots (axillary buds produced on current season’s floral structures) die. This usually occurs on floral structures that fail to set fruit. Remaining floral structures bear fruit and produce axillary buds that do not go through a vegetative or resting cycle but instead flower in the following season. This results in regular annual flowering on sequential terminal buds, which is termed the “bourse-over-bourse” phenomenon (Lespinasse and Delort, 1993; Lauri et al., 1995; Lauri

et al., 1997). Artificial spur extinction (ASE) is a thinning method which imitates this mechanism by manually removing buds between late dormancy and early budbreak (Lauri and Lespinasse, 1999, 2000; Lauri et al., 2004; Tustin et al., 2012). In 'Granny Smith', using ASE to reduce the density of floral buds and reducing the number of flowers within retained flower clusters to one increased the proportion of buds setting fruit (Lauri and Terouanne, 1999). In 'Scifresh' (Jazz™) and 'Gala', reducing the floral bud density using ASE without flower thinning increased fruit set in the remaining floral buds (Tustin et al., 2011a; Tustin et al., 2012; Breen et al., 2014). In all genotypes, it was hypothesised that removal of buds through ASE reduced competition for limited resource supply among remaining sinks, thereby improving fruit set on retained buds.

The earlier in the reproductive process and greater degree to which reproductive sink size is reduced by flower cluster thinning or early fruitlet thinning, the greater the change observed in the remaining sinks, such as increased fruit or vegetative growth and return bloom (Quinlan and Preston, 1968; McArtney et al., 1996; Byers, 2003; Embree et al., 2007).

From a horticultural point of view, ASE has been suggested to be an especially useful and reliable form of very early thinning (Tustin et al., 2012). However, physiologically, ASE may differ considerably from conventional thinning as it not only removes some reproductive sinks as conventional thinning does, but also removes a portion of vegetative sinks, reduces leaf area, and is imposed earlier in the season. Consequently, ASE provides a useful method to improve understanding of the physiological processes affecting fruit set, quality and productivity. We compared 'Royal Gala' apple trees thinned using ASE, with trees thinned by flower cluster thinning (FCT), and trees thinned by fruit removal after natural fruit drop (Control), using a range of thinning densities (crop loads). We hypothesised that early reduction in sink competition in ASE and FCT treatments would increase fruit set and harvest mean fruit weight (MFW) compared with the Control, and that increased leaf area afforded by FCT would improve fruit set and MFW compared with ASE.

Materials and Methods

In winter 2011, 'Royal Gala'/'M9' trees of similar size and form were selected on a Plant & Food Research orchard in Motueka, Nelson, New Zealand. The block was planted in 2006 at 3.7 m x 1.25 m. Trees had been pruned to a Central Leader Tall Spindle system since planting. Where necessary, from 2011, every winter, limbs were removed to standardise the number of limbs in each

tree to six to seven limbs per metre of effective canopy height and remaining limbs were tied down to 10–15° below the horizontal to reduce vigour. Trees were randomly allocated as single whole-tree plots within four blocks using a randomised complete block design containing two factors: three methods of thinning (artificial spur extinction (ASE), flower cluster thinning (FCT) and post fruit drop hand-thinning (Control, C)) and three levels of floral bud density (2, 4 or 6 buds or clusters.cm⁻² basal branch cross-sectional area (BCA)). ASE treatments were established one season prior (spring 2011–12) to FCT and Controls to ensure that during the first season of data collection (2012–13) floral bud initiation had been established under ASE conditions. In 2011, trees assigned to FCT and Control treatments were hand-thinned after final fruit drop to a commercial crop load. In the following two seasons (2012–13 and 2013–14) all treatments were fully applied. Each treatment was applied to the same tree each season. Trees were managed under conventional spray and irrigation practice, and pollination was achieved through introduction of commercial bee hives.

Summation of the individual BCAs for each tree showed that at a floral bud density of 5 buds.cm⁻² BCA, a single fruit per bud would achieve approximately the same number of fruit per tree as the commercial target for the orchard. ASE was applied between dormancy and early budbreak every season, at either two (ASE2), four (ASE4) or six (ASE6) buds.cm⁻² BCA. ASE was applied at a branch unit level, by calculating the number of floral buds to be retained on each branch and removing all other buds by hand. Small buds, buds in shaded areas of the canopy, and those on the underneath of branches were preferentially removed, retaining buds spaced out along the length of each limb. Where possible, terminal buds on short, 10 to 15 cm length shoots were retained, as these have been shown to produce larger fruit than other buds (Breen et al., 2007; Tustin et al., 2011b). FCT was applied when most floral clusters in the block were at 'pink' stage (between 'tight cluster' and very early 'bloom' (Chapman and Catlin, 1976)). The selection criteria for retention/removal of floral clusters in FCT were the same as for retention/removal of buds in ASE. Whereas in ASE whole buds were removed, in FCT, final floral bud densities were achieved by only removing flower clusters in unwanted buds, leaving the primary spur leaves and developing bourse shoot undamaged. Control trees were not thinned until after natural fruit drop was complete (end November) and fruit set had been recorded. No chemical thinners were applied to any treatments, allowing determination of natural fruit set responses.

One-year lateral buds on 'Gala' produce smaller fruit than spur or terminal buds (Volz et al., 1994). Consequently, on ASE, one-year lateral buds were removed, apart from one every 5–7 cm, which was de-blossomed to provide additional choice of floral sites the following season. On FCT, all one-year lateral buds were de-blossomed. On Controls, fruit was removed from one-year laterals after recording fruit set. Fruit set was recorded after final fruit drop and prior to setting final crop loads. Final crop loads were set by hand-thinning. The same criteria for retention of buds used in ASE were used to select clusters to retain fruit in Controls. Over all thinning methods, where fruit was held on 4 or 6 buds.cm⁻² BCA, these were thinned to 4 and 6 fruit.cm⁻² BCA respectively, with a single fruit per bud. Treatments where fruit was held on 2 buds.cm⁻² BCA were thinned to 4 fruit.cm⁻² BCA, leaving two fruit per bud. On all treatments, where necessary, two fruit per bud were retained to achieve target fruit densities.

Flowering and fruit set responses were recorded on the same three representative mid-canopy limbs in each tree over two seasons (2012–13 and 2013–14). At flowering and prior to removing flowers in FCT and on one-year laterals, the numbers of spur, terminal and one-year lateral floral buds were recorded for each limb. Fruit set was not recorded on Control 2 and 4 treatments in 2012–13 as all Control trees had had similar observed floral load and set crop load the previous season. After natural fruit drop was complete and before hand-thinning, the number of fruit set on each floral bud was recorded, including buds that failed to set fruit. In FCT, the number of floral buds with fruit was checked against recorded number of floral buds retained after flower cluster thinning, to calculate, by difference, the number of floral buds that set no fruit.

Floral bud numbers on each limb were converted to spur + terminal (S+T) and total (S+T + lateral) floral bud density using BCA. Fruit set data were analysed in two ways. Firstly, to investigate within-bud fruit set patterns, the number of S+T buds in each of seven classes of fruit set (0–6 fruit per bud) was converted to their proportion of the total floral bud number for that limb. Secondly, fruit set density (number of fruit.cm⁻² BCA) and mean fruit set per floral bud was calculated for each limb. Data from these three limbs were then used to calculate tree means and treatment means in each season. Fruit maturity was monitored using commercial criteria for starch pattern index (SPI, ENZA International Ltd, 8-point chart) and background colour (BGC, ENZA International Ltd, 10-point chart) to determine four sequential commercial harvesting dates in 2013 and a mid-point harvest date in 2014. At harvest 2 in 2013 and mid-point harvest in 2014, a randomly selected 10 fruit sample of

export grade fruit with BGC 4-5 was collected for each plot and assessed for fruit weight, flesh firmness, SPI and dry matter content. Individual fresh weights of all other fruit were recorded on a plot basis to calculate yield.

For most data, plot mean values were subjected to ANOVA procedures in Genstat v14 (VSN International Ltd, UK) using a significance of $P \leq 0.05$. Means separations were conducted using least significant differences at 5%. Where distribution of residual values did not appear normal, data were log transformed to stabilise variance and where necessary a small positive number (about half of the smallest observed value) was added to all numbers to remove zeros, and removed after back-transformation. Regression analyses were conducted using procedures in Genstat v14 to test whether the relationships between floral bud density and mean fruit set per bud, and floral bud density and fruit set density were similar among treatments. To do this, models with a common line, parallel lines or separate lines per treatment were compared. To fit curves, the most appropriate curve was chosen from a set of standard curves on the basis of residual plots and adjusted R-squared values.

Results

After normal maintenance winter pruning in 2012, all trees were of similar size; mean values for trunk cross-sectional area (18.3 cm^2), the number of branches per tree (18.2) and the sum of the individual BCAs (47.2 cm^2) did not differ among treatments.

In both seasons, actual S+T floral bud densities achieved in Control and FCT trees prior to flower cluster thinning were in the range 10 to $14 \text{ buds.cm}^{-2} \text{ BCA}$ (Table 1). Although there was some natural variation in these values, the previous season's crop load did not affect floral bud density.

Bud removal in ASE and flower cluster removal in FCT significantly reduced floral bud density so that within each target bud density, FCT and ASE did not differ from each other or from target bud densities (Table 1). Prior to their removal, lateral bud floral density was greater in ASE trees than Controls. In FCT in 2012, lateral bud floral density was more similar to Controls than ASE while in 2013 the converse was true. Retaining flowers on lateral buds in the Controls resulted in three to four times greater total floral bud density than in ASE and FCT.

The overall response curve of mean number of fruit set in S+T buds to S+T floral bud density (Figure 1 insets) was slightly higher in 2012 than in 2013. More rain fell over the 21-day flowering period in

2013 (145 mm) compared with 2012 (33 mm), and during the 2 to 4 weeks after bloom, cloudy and rain days also reduced total radiation in 2013 (311 MJ.m⁻²) compared with 2012 (386 MJ.m⁻²).

Mean fruit set per floral S+T bud for all thinning methods in both seasons was well described by power functions (Figure 1). Mean fruit set per floral bud in S+T buds declined from three to four fruit per bud at a floral bud density of two, to approximately half of this at floral bud densities of 10 to 18. Among different thinning methods within each season, power functions with differing constant values (horizontal asymptotes) accounted for more than 70% of the variance in the data. These functions showed that in both seasons FCT set an average of approximately 0.6 more fruit per floral bud than ASE (P=0.009 and 0.011 in 2012 and 2013 respectively).

Table 1. The effect of three methods of thinning (artificial spur extinction (ASE), flower cluster thinning (FCT) and post fruit drop hand-thinning (Control)) at three target floral bud densities (FBD, 2, 4 and 6 buds.cm⁻² branch cross-sectional area) on actual floral bud density of 'Royal Gala' trees immediately prior to flowering in New Zealand in 2012 and 2013. Floral bud densities in FCT treatments were recorded before (pre) and after (post) application of the treatment. LSDs for means of Total FBD may be used to compare with those of Spur and Terminal FBD in ASE and FCT treatments.

Main effect		2012			2013		
Thinning method	Target FBD	2	4	6	2	4	6
Spur + Terminal FBD							
ASE		2.0	4.6	6.4	2.9	3.9	6.0
FCT post		2.8	4.0	5.7	2.5	4.3	6.2
FCT pre		10.8	9.9	9.7	12.2	12.4	12.0
Control		11.7	13.3	12.5	10.8	14.2	13.9
P-value; LSD5% ^a		<0.001; 1.30		<0.001; 1.31			
Lateral FBD							
ASE ^b		7.4	7.5	7.9	8.7	7.2	6.4
FCT pre ^b		3.2	1.8	3.8	6.6	5.2	5.5
Control		3.8	4.5	5.7	1.8	2.7	3.4
P-value; LSD5% ^a		<0.001; 2.56		<0.001; 2.72			
Total FBD							
ASE		2.0	4.6	6.4	2.9	3.9	6.0
FCT		2.8	4.0	5.7	2.5	4.3	6.2
Control		15.5	17.8	18.2	12.6	16.9	17.3
P-value; LSD5% ^a		<0.001; 1.29		<0.001; 1.28			

^a LSDs presented are ratios (i.e. differences on the log scale, back-transformed); when comparing two means, if the larger is more than the ratio times the smaller, then the difference is significant.

^b Flowers on ASE and FCT lateral buds were removed immediately after counting.

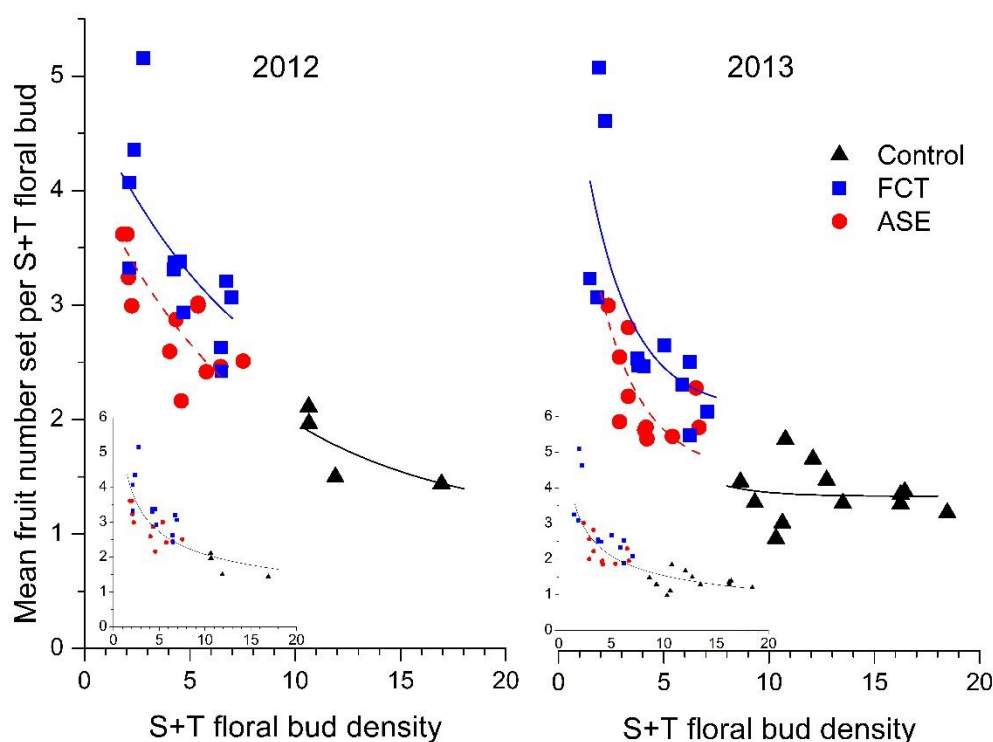


Figure 1. The effect of spur and terminal (S+T) floral bud density (number of buds.cm⁻² branch cross-sectional area) on mean number of fruit that set per floral spur or terminal bud in 'Royal Gala' trees after final fruit set in New Zealand in 2012 and 2013. Trees were thinned using post fruit drop hand-thinning (Control), flower cluster thinning (FCT) or artificial spur extinction (ASE). Lines of fit for 2012 are: Overall response (inset), ($R^2=0.60$), $y=0.52+3.87*0.91^x$; Individual treatment responses ($R^2=0.71$), Control, $y=1.08+3.24*0.88^x$; FCT, $y=1.57+3.24*0.88^x$; ASE (broken line), $y=0.96+3.24*0.88^x$. Lines of fit for 2013 are Overall response (inset), ($R^2=0.66$), $y=1.36+4.21*0.71^x$; Individual treatment responses ($R^2=0.73$): Control, $y=1.35+4.09*0.62^x$; FCT, $y=2.10+4.09*0.62^x$; ASE (broken line), $y=1.55+4.09*0.62^x$.

Both thinning method and target bud density significantly affected fruit set (Table 2). In both seasons Control trees set fewer fruit per floral bud than other thinning methods. In both seasons FCT set slightly more fruit per floral bud than ASE. Within thinning methods that manipulated floral density (ASE and FCT), there was a trend for a decline in the numbers of fruit set per floral bud as target floral bud density increased (Table 2). Bud Density 2 set more fruit per bud than densities 4 or 6. However, in 2012 this response was much greater in FCT than in ASE, and in 2013, a significant

interaction of main effects ($P=0.041$ S+T, $P=0.039$ Tot), showed that there was no response in ASE (data not shown). Trends for higher mean numbers of fruit per bud at a bud density of four compared with six were not significant. In Control trees, where floral density was not manipulated, the number of fruit per bud was consistently lower than treatments where floral bud density was set to 2, 4 or 6 buds.cm⁻² BCA.

Table 2. The effect of thinning method (artificial spur extinction (ASE), flower cluster thinning (FCT) and post fruit drop hand-thinning (Control)) and bud density (2, 4 and 6 buds.cm⁻² branch cross-sectional area) on the mean number of fruit set per floral spur + terminal (S+T) bud (MFB) in 'Royal Gala' trees in New Zealand after completion of fruit drop, but prior to hand-thinning in 2012 and 2013. Means for thinning method are means over all bud densities. Bud density two, four and six are means of ASE and FCT and are compared with mean for all bud densities of the Control (thinned afterwards).

Year	2012	2013		2012	2013
Thinning method	S+T MFB	S+T MFB	Bud density	S+T MFB	S+T MFB
ASE	2.9	2.2	2	3.8	3.3
FCT	3.4	3.0	4	3.0	2.3
Control	1.8	1.4	6	2.7	2.2
			Control	1.8	1.4
P-value	<0.001	<0.001	P-value	<0.001	<0.001
LSD5%	0.48	0.63	LSD5% ^a	0.51	0.68
			LSD5% ^b	0.42	0.54

^a LSD for comparison among 2,4 and 6. ^b LSD for comparison of Control with 2, 4 or 6.

In both 2012 and 2013, in Control trees the greatest proportion (30–40%) of floral S+T buds set a single fruit and the proportions of buds setting multiple fruit declined with increasing fruit number per bud (Figure 2A, B). The proportion of buds setting zero fruit varied between seasons from 8% in the 2012 season to 27% in the 2013 season.

Greatly reduced floral bud densities in ASE and FCT compared with the Control significantly altered the proportion of buds in the 0 to 6 fruit per bud categories (Figure 2A, B). In 2013, when 27% of floral buds in the Control set zero fruit, in both ASE and FCT this proportion was significantly reduced. Consequently, with ASE and FCT, the proportion of buds setting zero fruit was low and more consistent between seasons than the Control. ASE and FCT also resulted in a lower proportion of

buds setting a single fruit and, in 2012, the proportion setting two fruit. Lower proportions of floral buds setting zero to two fruit were associated with greater proportions setting three to five fruit. In general, fruit set patterns suggested that FCT set more fruit in clusters of three to five than ASE, however these differences were never significant.

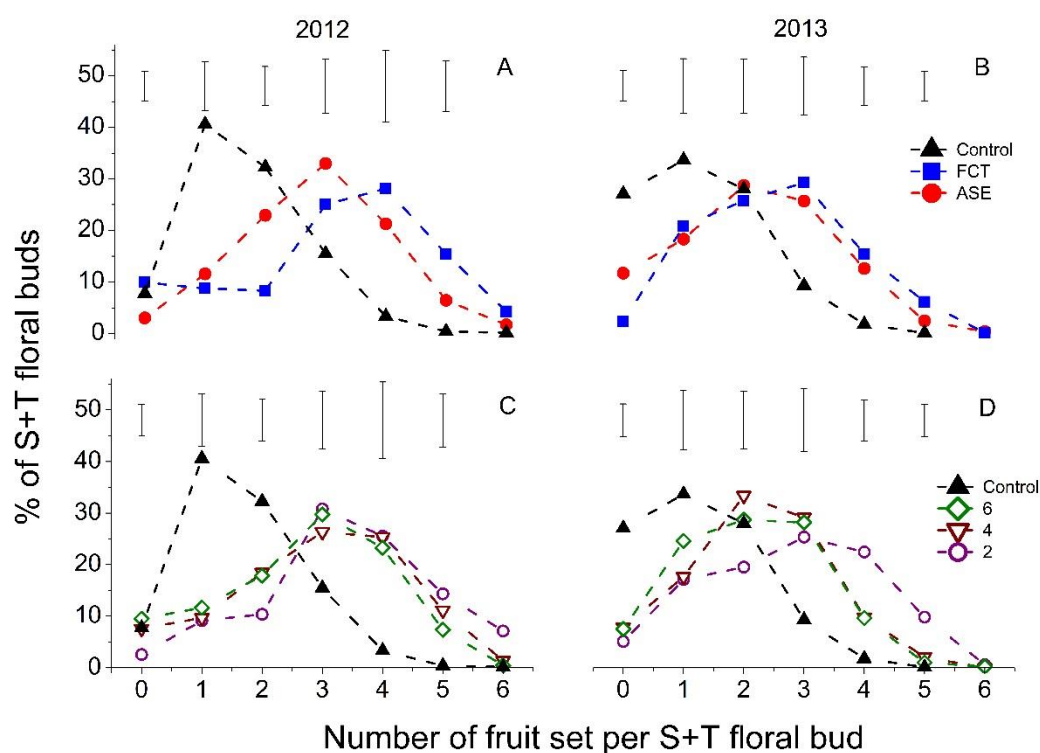


Figure 2. Within-bud fruit set responses of spur and terminal (S+T) floral buds (percentage of buds setting zero to six fruit per bud) on 'Royal Gala' trees in New Zealand after final fruit set in 2012 and 2013 (Plots A and B; main effect of each thinning method: post fruit drop hand-thinning (Control), flower cluster thinning (FCT) or artificial spur extinction (ASE). Plots C and D; main effect of each floral bud density in ASE and FCT (6, 4 and 2 buds.cm⁻² branch cross-sectional area) compared with all bud densities in Control (thinned afterwards). Floral bud densities in Control trees are given in Table 1. Error bars are LSDs at 5%. Data for buds setting six fruit were not analysed because of high numbers of zeros.

Thinning to 6, 4 and 2 floral buds.cm⁻² in FCT and ASE increased within-bud fruit set compared with the Control (Figure 2C, D). Within-bud fruit set did not differ between floral bud densities four and six. Floral bud densities of four and six showed trends for higher proportions setting zero and two fruit per

bud and lower proportions setting four to six fruit compared with floral bud density two, but these differences were only significant in 2013.

S+T fruit set density increased with increasing floral bud density (Figure 3 insets) and was higher in 2012 than 2013 apart from at very low floral bud densities. Floral bud densities in the Control did not overlap those of FCT and ASE and in neither year did fruit densities exceed 25 fruit.cm⁻². In both seasons, FCT produced small (1.6 to 2.6 fruit.cm⁻²) but significantly (P=0.002) greater fruit densities than ASE over the measured FBD range.

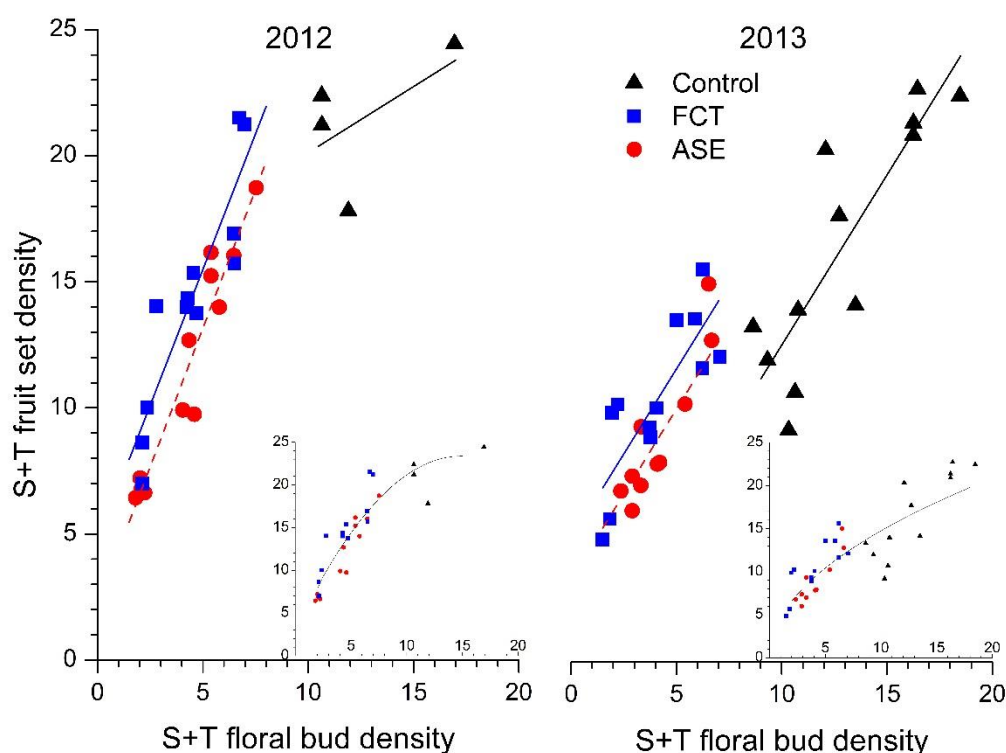


Figure 3. The effect of spur and terminal (S+T) floral bud density (number of buds.cm⁻² branch cross-sectional area (BCA)) on the density of fruit set on spur and terminal buds (number of fruit set.cm⁻² BCA) in 'Royal Gala' trees after final fruit set in New Zealand in 2012 and 2013. Trees were thinned using post fruit drop hand-thinning (Control), flower cluster thinning (FCT) or artificial spur extinction (ASE). Lines of fit for 2012 are: Overall, ($R^2=0.82$), $y=-0.09x^2+2.73x+3.01$; Individual ($R^2=0.88$), Control, $y=14.87+0.53x$; FCT, $y=4.73+2.15x$; ASE (broken line), $y=2.18+2.20x$. For 2013 Overall, ($R^2=0.74$), $y=4.66x^{0.5003}$; Individual ($R^2=0.84$): Control, $y=-0.91+1.35x$; FCT, $y=4.89+1.35x$; ASE (broken line), $y=3.30+1.35x$.

Crop load and fruit density at harvest varied slightly among thinning methods (Table 3). Regression analysis (not shown) revealed that differences in mean fruit weight between ASE and FCT in 2014 were the consequence of these small differences in crop load and fruit density, not thinning method. In both seasons, reduced mean fruit weight of 18–32g in the Control compared with ASE and FCT were independent of crop load.

Bud densities 2 and 4, both set to 4 fruit.cm⁻² at hand-thinning, did not differ in at-harvest crop load, fruit density or yield. Regression analysis (not presented) showed that greater mean fruit weight (MFW) recorded in bud density 2 than 4 in 2014, was the effect of slight variation in crop load and fruit density. Bud density of 6 had a greater crop load and fruit density than other bud densities and this increased yield by 10–20 t.ha⁻¹ at the expense of 10–30g of weight per fruit.

Table 3. At-harvest (2013 and 2014) crop load (fruit number.cm⁻² trunk cross-sectional area, TCA), fruit density (fruit number.cm⁻² branch cross-sectional area, BCA), mean fruit weight (MFW) and calculated yield of 'Royal Gala' trees grown in New Zealand treated with three thinning methods (artificial spur extinction (ASE), flower cluster thinning (FCT) and post fruit drop hand-thinning (Control)) applied at three floral bud densities (2, 4 and 6 buds.cm⁻² BCA) to set fruit densities of 4 and 6 fruit.cm⁻² BCA.

Main effect	2013				2014			
	Crop Load (frt.cm ⁻² TCA)	Fruit Density (frt.cm ⁻² BCA)	MFW (g)	Yield (t.ha ⁻¹)	Crop Load (frt.cm ⁻² TCA)	Fruit Density (frt.cm ⁻² BCA)	MFW (g)	Yield (t.ha ⁻¹)
Thinning method								
ASE	12.5	4.7	189.7	75.8	11.0	4.5	187.4	83.5
FCT	13.1	5.0	185.6	77.8	14.2	5.3	175.2	92.6
Control	13.8	5.5	168.1	73.4	14.0	5.4	155.5	78.7
P-value	0.269	0.028	<0.001	0.764	0.009	0.005	<0.001	0.109
LSD5%	-	0.64	5.83	-	2.14	0.55	8.04	-
Bud/Fruit Density								
2/4	10.9	4.1	188.4	64.0	9.9	3.9	186.9	74.9
4/4	12.1	4.7	183.3	76.0	11.0	4.4	178.4	83.4
6/6	16.4	6.4	171.8	87.0	18.2	6.9	153.0	96.4
P-value	<0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	0.009
LSD5%	1.70	0.64	5.83	12.37	2.14	0.55	8.04	13.14

All fruit used in laboratory assessment were of similar maturity, as starch pattern index did not differ among treatments (Table 4). There were small (0.4 kgf) significant differences among main effects on

fruit firmness in 2014. Regression analysis (not shown) confirmed that significantly higher fruit dry matter content in FCT in 2013 was independent of crop load or fruit density, suggesting a real treatment effect, however this result was not repeated in 2014. The highest fruit density treatment (6 fruit.cm⁻² BCA) produced fruit with significantly lower (0.6–0.8%) fruit dry matter content than other fruit density treatments in both seasons.

Table 4. At-harvest (2013 and 2014) starch pattern index (SPI), fruit flesh firmness (FF) and dry matter content (DMC) on a sample of fruit removed from 'Royal Gala' trees grown in New Zealand treated with three thinning methods (artificial spur extinction (ASE), flower cluster thinning (FCT) and post fruit drop hand-thinning (Control)) applied at three floral bud densities (2, 4 and 6 buds.cm⁻² branch cross-sectional area, BCA) to set fruit densities of 4 and 6 fruit.cm⁻² BCA.

Main effect	2013			2014		
	SPI (0-7)	FF (kgf)	DMC (%)	SPI (0-7)	FF (kgf)	DMC (%)
Thinning method						
ASE	2.8	9.0	15.5	2.5	9.2	15.2
FCT	2.5	9.2	16.0	2.1	9.4	15.1
Control	2.7	9.2	15.4	2.2	9.6	15.1
P-value	0.273	0.138	0.008	0.174	0.017	0.796
LSD5%	-	-	0.42	-	0.23	-
Bud/Fruit Density						
2/4	2.6	9.1	15.9	2.4	9.4	15.3
4/4	2.5	9.3	15.8	2.1	9.6	15.3
6/6	2.8	9.0	15.1	2.5	9.2	14.7
P-value	0.258	0.119	<0.001	0.184	0.011	<0.001
LSD5%	-	-	0.42	-	0.23	0.32

Discussion

Carbohydrate limitation between two and four weeks after bloom is regarded as a major factor in determining fruit set and size in apple. In this study we hypothesised that reducing sink size at or before flowering using ASE or FCT treatments and reducing source size in ASE would result in altered fruit set and fruit growth compared with Control trees. Commercially, pre-fruit set thinning must achieve a strong and predictable fruit set response to ensure crop load targets are met.

In this study, fruit set density and number of fruit set by individual spur and terminal buds was strongly related to branch floral bud density. In both seasons there appeared to be a natural maximum fruit set capacity of 20–25 fruit.cm⁻² BCA expressed in Control trees, which had unmodified floral bud

densities of 11–18 buds.cm⁻² BCA. The upper limit to fruit set measured in these trees may be the result of early-season carbohydrate resource supply limitation. This natural fruit set resulted in 950–1200 fruit per tree, which were primarily set up as one or two fruit per bud. In commercial production, this fruit load would have to be reduced by 75–80% to achieve commercial targets of fruit size and quality. However, this low number of fruit per bud is not highly responsive to chemical thinning and also difficult to hand-thin, as numerous clusters with few green fruit are easily obscured by foliage. Controlled reduction of the density of flowering buds using ASE or FCT increased the probability that a floral bud would set fruit and increased the number of fruit that set per bud. Reducing the density of developing fruit buds may increase the availability of early-season carbon resources to remaining fruiting buds, resulting in increased fruit set within these buds. It is possible that this response may be partly the consequence of increased access to re-mobilised stored reserves within the limb. In ASE, bud thinning may increase availability of reserves for development of remaining buds during budbreak when stored reserves appear to play their primary role (Hansen, 1971; Lakso et al., 2006b). However, compared with ASE, FCT was applied later, at ‘pink bud’ stage when the contribution of stored reserves is considered to be largely complete, and yet mean fruit set per bud in FCT was generally greater than in ASE. Consequently, the contribution of stored reserves to increasing within-bud fruit set in response to ASE and FCT cannot explain this response.

It is important to consider that Control trees are not directly equivalent to FCT and ASE as they retained additional floral buds including one-year-old lateral floral buds and, compared with ASE, additional vegetative buds. The presence of fruiting lateral buds would have increased competitive demand for carbon resources, and removing these in ASE and FCT may have contributed to increased within-bud fruit set in these treatments. However as differences in lateral floral bud density in Control trees did not appear to affect S&T fruit set response (data not shown), the absence of lateral flower buds cannot sufficiently explain altered within-bud fruit set in ASE and FCT. In ASE and FCT, large spur and terminal buds in well-lit areas of the canopy were retained for fruiting whereas in Control trees there was no fruiting site selection until hand thinning. This may have positively influenced fruit set in ASE and FCT irrespective of other treatment effects.

By petal fall, primary and spur leaves contribute a significant portion of their photosynthate to recently fertilised fruit (Tustin and Lai, 1990) and non-fruiting spurs are able to export carbohydrates to fruiting spurs (Ferree and Palmer, 1982; Palmer et al., 1991; Corelli Grappadelli et al., 1994). Using FCT,

retaining the same leaf area as Control trees, but reducing floral bud density by 50–80% theoretically greatly increased the leaf area per floral bud uniformly over all branches. Consequently, the availability of photosynthates to remaining floral buds may have been increased, leading to increased within-bud fruit set.

One of the most interesting responses in this study is that within-bud fruit set in ASE was only marginally lower than in FCT, despite presumably vastly reduced leaf area. Fruit set and development are highly dependent on light interception, especially that of the spur canopy (Kondo and Takahashi, 1987; Byers et al., 1991; Wünsche and Lakso, 2000). Breen et al. (in press) have recently shown that ASE4 and ASE6 treatments in 'Gala' removed up to two thirds of S+T floral buds compared with Control trees. Despite this, canopy light interception was not decreased in ASE6, and in ASE4, light interception was only reduced by 2% and only until 4 weeks after bud break (WABB) (well before petal fall). Even with ASE2, light interception was only 5–7% lower than Controls and was not different by 7 WABB, about 2 weeks after petal fall. These surprisingly small differences in light interception between Controls and ASE show that canopy light interception is maximised at bud densities as low as 4–6 buds.cm⁻² BCA, suggesting there is no loss of carbon assimilation potential of the canopy at these bud densities. Modelling of structure and light interception parameters in Centrifugally Trained (CT) trees (in which bud density is reduced using ASE) compared with Central Leader trees has shown light interception of the whole canopies did not differ at full canopy, but leaf irradiance on fruiting shoots was greater in CT trees (Stephan et al., 2008). On FCT and Control trees, it is likely that at the natural, higher bud densities, developing foliage created shading among buds and their associated leaves very early in the season. Removal and uniform spatial distribution of buds in ASE may have reduced this shading and improved light transmission within the canopy. In this situation, individual fruit bearing spurs may be exposed to an improved light environment, with individual leaves having greater duration of light exposure. As net carbon exchange on a leaf area basis is known to show a positive curvilinear response to crop load (Palmer et al., 1997), increased light exposure and carbon exchange may compensate for reduced leaf area in ASE.

Consequently, the mechanisms by which within-bud fruit set was increased by FCT probably differed from ASE. In FCT, the mechanism may be that reduction in floral bud density reduced the density of carbon sinks and increased the availability of current photosynthate to remaining buds resulting in increased fruit set. Reduction in floral bud density from FCT6 to FCT2 resulted in further increases in

within-bud fruit set, suggesting that availability of photosynthate to remaining buds was increased as the density of flowering buds decreased. In ASE, the mechanism may be that increased leaf irradiance within the floral spur increased photosynthate supply within the spur and resulted in increased fruit set. Reduction of floral bud density from ASE6 to ASE2 did not greatly reduce canopy light interception or improve within-spur leaf irradiance and therefore did not alter within-bud fruit set appreciably.

Even at very low floral bud densities, within-bud fruit abscission still occurred. In part, this is likely the result of abscission of damaged or unfertilised flowers. However, carbon balance modelling of 'Gala'/'M9' trees in Geneva, New York has shown that even at fruit numbers as low as 300 fruit per tree, in some seasons there may be periods where photosynthesis cannot supply carbon demand from developing organs, resulting in fruit abscission (Lakso et al., 2006b). In this study, at flowering, trees with the lowest floral bud densities (ASE2 and FCT2) had estimated total flower numbers of ~470 per tree. Consequently, even in these trees with low bud numbers, pruned to have open canopies, and with the high incident light environment characteristic of New Zealand (Palmer et al., 2002), early-season photosynthesis may not have sufficiently supplied carbon demand, and thus limited fruit set. Alternatively, despite improved carbon availability, developing shoots still maintained sink priority over developing fruit which limited fruit set.

Final fruit size is largely determined by cell number (Lakso et al., 1995). Early competition among fruit for resources has been shown to reduce individual fruit growth and final fruit fresh weight by limiting fruit cell division (Lakso et al., 1995; Jackson, 2003). In the cell division phase, the earlier that thinning is carried out, the greater the positive effect on individual fruit weight. If thinning occurs early enough (before bloom), yield of thinned and unthinned trees can be similar because the gain in fruit weight of retained fruit compensates for reduced fruit numbers (Jackson, 2003). In this study the effect of reducing crop loads from 6 to 4 fruit.cm⁻² BCA was apparent in the 10–30g increase in MFW and 0.6–0.8% increase in fruit DMC. However, in this case, the increase in MFW and DMC did not compensate for reduced fruit numbers and consequently yield was reduced by 11–23t.ha⁻¹. FF was largely unaffected by thinning method or the range of crop loads tested.

The same carbon balance factors proposed to explain increased within-bud fruit set in FCT and ASE may also explain difference in fruit size among treatments. In Control trees there was no intervention to adjust inter-fruit competition until a stage when cell division was largely complete (Blanpied and

Wilde, 1968; Jackson, 2003; Tromp and Wertheim, 2005). In both FCT and ASE, carbon availability to young fruit was probably improved over Control trees, resulting in increased cell division and expansion, producing larger fruit at harvest. Compared with Control trees, in FCT, fruit density was reduced early, increasing the availability of photosynthates to remaining fruit, especially during the cell division phase, and resulting in fruit of ~20g greater mean fruit weight. For the same reason, mean fruit weight increased with reduced fruit density as flower cluster densities in FCT were reduced from FCT6 to FCT2 (main effects shown in Table 3, interactions NS). In ASE, the improved irradiance to individual fruit-bearing spurs probably increased photosynthate production within those spurs, leading to an increase in MFW of 22–32g. In contrast to within-bud fruit set however, decreasing floral bud density from ASE6 to ASE2 continued to increase MFW. Although total canopy light interception from 7WABB does not differ among ASE treatments (Breen et al., in press), this result suggests that total photosynthate availability within each spur continued to be improved at lower floral bud densities. A number of factors may have contributed to this. Improved irradiance of spurs of lower FBD after full canopy development or increased leaf area as a result of greater bourse shoot development may have increased carbon supply during cell enlargement. However, it is also possible that a small increase in irradiance or reduction in competition for stored carbon may have resulted in a small increase in fruit cell division which did not affect fruit set but did increase final fruit size.

In both seasons flowering and fruit set was greater than needed to produce a full commercial crop and the slightly lower overall fruit set in 2013 was of little practical consequence. The cause of this slight difference may have been reduced pollination through more rainfall reducing bee activity over flowering, or extended cloudy periods reducing photosynthesis and exacerbating carbohydrate supply deficit in the 2–4 WAB period (Lakso et al., 1998; Lakso et al., 1999).

In apples, current commercial practice relies primarily on chemical and hand-thinning to reduce fruit numbers and achieve target crop load and fruit size. The unpredictability of chemical thinner response requires that a number of applications are made which are followed by hand-thinning to reach the target crop load. As a result, some of the advantage of early reduction in crop load is lost. Alternative methods of thinning such as mechanical blossom thinning and self-thinning scion genotypes may provide useful alternatives to chemical thinning (Celton et al., 2014; Schupp and Kon, 2014). Thinning of flower clusters and flowers within clusters is an established commercial practice in some regions to improve fruit size and prevent alternate bearing in cultivars such as 'Fuji' (Koike et

al., 2003). Ideally a method of very early thinning is sought that has predictable outcomes and moderate costs, or at least costs that may be offset by gains in other areas. In this study, in Control trees, the overall lower mean fruit number per floral bud in 2013 was primarily the consequence of a much greater proportion of buds failing to set fruit. Removal of half or more of the floral and fruiting S+T sinks either by removing the whole bud in ASE, or flowers alone in FCT, reduced this proportion by over 50% to a level very similar to 2012. As discussed by Breen et al. (2014) and Tustin et al. (2012) in respect of ASE, this response shows that removal of a portion of the competing floral sinks very early, whether through ASE or FCT, greatly improves the predictability that the remaining buds will set one or more fruit. In this study, this response was reasonably stable between seasons, without the use of chemical thinners, even when factors affecting natural fruit set such as low light and pollination varied.

Greater mean fruit weight in ASE and FCT at the same crop loads as the Controls is also horticulturally important as an increase in weight of fruit in this size range is closely related to increased crop value. Although yields did not differ significantly among treatments in this research, mean yields for ASE and FCT tended to be larger than the Control, demonstrating the physiological effects of early thinning and improved irradiance.

Conclusions

Early-season removal of carbon sinks in ASE and FCT increased fruit set in individual buds and increased harvest mean fruit weight compared with the Control. Unexpectedly, much greater early-season leaf area in FCT produced only a small increase in fruit set over ASE and there were no conclusive differences in harvest MFW, fruit DMC or FF.

The mechanism by which fruit set and fruit growth is altered may differ between FCT and ASE. Compared with Controls, reduced flowering bud density in FCT reduced the density of developing organs without altering leaf area. This may have relieved carbon shortage, resulting in increased within-bud fruit set and harvest MFW. In ASE, the response was similar to FCT but the treatment removed both floral buds and leaf area. In this treatment, early-season carbon availability to remaining buds is thought to have been improved through increased photosynthate production by fruit-bearing spur leaves as a result of improved within-canopy irradiance.

Considering the various models proposed to explain apple fruit abscission during post-bloom drop, it appears that limitations to carbon supply during this period reduce growth rate in some fruit (Greene et al., 2005; Lakso et al., 2006a), possibly those which had lower fertilisation success or later development (Bangerth, 1989; Tromp and Wertheim, 2005). If this carbon (particularly sugar) supply to the fruit cortex drops low enough for a sustained period, sugar signalling inhibits further embryo development, stalling basipolar auxin flow and triggering abscission (Bangerth, 1989; Botton et al., 2011; Eccher et al., 2014).

In the research presented here, compared with the Controls, both FCT and ASE increased the predictability of fruit set through reduced seasonal variability in the proportion of buds that did not set fruit. Although FCT did increase fruit set over ASE, the increase was in multiple fruit per bud, not singles. As a single fruit per cluster is the commercial target, FCT produced no commercial benefit over ASE. ASE is applied to trees in late dormancy, a period when labour demand is low and manipulation of the tree canopy is easy as foliage is absent. The process of bud removal is also much faster and simpler than flower cluster thinning. Consequently ASE presents a number of advantages over FCT and shows strong promise as a commercial thinning method.

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Chapter 4. Artificial Spur Extinction Alters Light Interception by 'Royal Gala' Apple Trees

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Abstract

Total dry matter production by apple orchards is positively related to light interception. Consequently, maximising light interception is important in commercial apple orchards, as it directly affects tree growth and yield. Artificial spur extinction (ASE) is a method of crop load control that reduces the density and alters distribution of floral buds in whole trees. Because ASE reduces total bud numbers on the tree, total light interception may be affected. The objective of this study was to investigate the impact of ASE treatments on canopy light interception. In a mature 'Royal Gala'/'M9' orchard in Hawke's Bay, New Zealand, we compared unmodified trees with trees managed using ASE. Bud densities in ASE trees were set to 2, 4 and 6 buds.cm⁻² branch cross-sectional area (BCA) in late winter, while unmodified trees were not altered. On both ASE and unmodified trees, crop loads were set after final fruit drop by hand thinning to two fruit per bud on 2 buds.cm⁻² BCA or single fruit on 4 and 6 buds.cm⁻² BCA. Over one season, fractional light interception by the canopies was calculated from the difference between the mean of irradiance readings above the canopy and the mean of irradiance readings below the canopy. Light interception by unmodified trees increased from ~30% at 2.5 weeks after budbreak (WABB) to ~60% at 8WABB and thereafter did not change until leaf-fall. Prior to 8WABB, light interception by trees set at ASE6 did not differ from that by the unmodified

trees. In trees set at ASE4 and 2, light interception was initially lower (25.7% and 22.7% respectively) than other treatments and this effect lasted until 5WABB in ASE4 and 8WABB in ASE2. At full canopy, trees managed with ASE intercepted ~4% more light than unmodified trees. Higher light interception of ASE trees is probably because ASE stimulates a higher proportion of fruiting spurs to produce short- to medium-length annual bourse shoots with greater leaf area than spur bourse buds.

Keywords

Malus x domestica Borkh, bud thinning, crop load, yield

Introduction

Both total dry matter production and the fruit (yield) portion of total dry matter production of apple orchards are linearly related to total seasonal light interception by the orchard canopy (Palmer, 1989). Choice of rootstock, orchard layout, pruning and training system (tree shape) play a critical role in light interception on apple orchards (Palmer, 1989). New Zealand's high radiation environment and long growing season contribute to the high yields achieved in this region (Palmer et al., 2002); well managed 'Gala' orchards at a planting density of 2400 trees.ha⁻¹ and a mid-season light interception of 50-55% can achieve 100 t.ha⁻¹ annually.

Artificial spur extinction (ASE) is a method of crop load control used to reduce density and alter distribution of floral buds in apple trees (Lauri et al., 2004; Tustin et al., 2012). Apart from improving fruit set (Tustin et al., 2011a; Tustin et al., 2012) and regularity of bearing (Lauri et al., 1995; Lauri et al., 1997), it is also used to improve light penetration into the canopy (Lauri et al., 2004). However, as ASE reduces total bud numbers on the tree, total light interception may be reduced and thus affect yield potential. To investigate the effect of ASE on total light interception, we compared ASE over a range of bud densities (crop loads) with similar crop loads set by hand thinning of unmodified trees. Among these treatments, the time-course of seasonal light interception was studied and correlated with pomological responses.

Materials and Methods

This study was conducted on a high yielding commercial 'Royal Gala'/'M9' orchard planted in 2005 at 3.4 x 1.25 m spacing, situated in Hawke's Bay, New Zealand. Trees had been pruned to a Central

Leader Tall Spindle system since planting and were managed under conventional practices with a target of 275 fruit.tree⁻¹ and 100 t.ha⁻¹. This orchard block, including experimental trees, received an annual dormancy-breaking spray in order to advance flowering date. The study was set up in winter 2011 in a randomised complete block design containing two factors: bud thinning (either unmodified or artificial spur extinction (ASE)), and fruiting bud density (fruit carried on either 2, 4 or 6 clusters.cm⁻² branch basal cross-sectional area (BCA)) in five blocks. Each plot consisted of nine adjacent trees: three adjacent trees in each of three adjacent rows. All plots were situated along a single set of three rows within a larger orchard planting, and a single treatment was set up in each plot. In winter 2012, the same treatments were re-applied to the same plots. In all treatments, the number of limbs in each tree was standardised to six to seven limbs per metre of effective canopy height, by removing limbs, particularly large basal limbs. Remaining limbs were tied down, if necessary, to 10-15° below the horizontal to reduce vigour. Other than this, all trees received normal commercial winter pruning each season.

After standardising limb numbers, the sum of the individual BCAs for each tree showed that using ASE, a bud density (number of buds per square centimetre of BCA) of about four would achieve approximately the same number of fruit per tree as the commercial target for the orchard. ASE was applied during late dormancy each season at bud densities of two (ASE2), four (ASE4) or six (ASE6) buds.cm⁻² BCA. ASE was applied on every branch individually by calculating the target floral bud number for that limb using the BCA and removing all other buds by hand. Weak buds, buds in shaded areas of the canopy, and those underneath branches were selectively removed, to leave buds spaced out along the length of each limb. Short shoots of 10 to 15 cm length with terminal buds were retained. Bud densities on unmodified trees were not altered. Lateral buds on one-year-old shoots on 'Gala' are generally avoided in commercial production as they produce smaller fruit than spur or terminal buds (Volz et al., 1994; Tustin et al., 2011b). In this experiment lateral buds on ASE trees were removed, apart from one every 5-7 cm which was de-blossomed to provide additional choice of floral sites in the following season. In all treatments, at flowering, three representative, mid-canopy limbs were selected, tagged and used to record individual limb floral bud density (FBD, number of floral buds.cm⁻² BCA). The calculated mean of these three FBDs provided an estimated tree mean. Chemical thinners were not applied to any treatment. All treatments were hand thinned to target crop loads on 21 November in 2012, after final fruit drop. Crop loads were set at a branch unit level.

ASE2, 4 and 6 treatments were set to 4, 4, and 6 fruit.cm⁻² BCA respectively. Buds on ASE2 treatments were thinned to carry 2 fruit per bud (2 fruit.bud⁻¹ x 2 buds.cm⁻²) to achieve a crop load appropriate to commercial loads and prevent excessive tree vigour. Buds on ASE4 and 6 treatments were thinned to 1 fruit per bud, but carried 2 fruit per bud in a small number of cases to compensate for floral buds that failed to set fruit (1 fruit.bud⁻¹ x 4 or 6 buds.cm⁻²). Unmodified trees were set up similarly. On Unmod2 treatments, fruit were removed to achieve 2 fruit per bud on 2 buds.cm⁻² BCA, while Unmod4 and 6 trees carried a single fruit per bud on 4 and 6 buds.cm⁻² BCA respectively. Fruit on lateral buds of one-year-old shoots were removed.

To allow the trees to adjust their growth habit to altered canopy structure, light interception was not measured until the second (2012-13) season. Light interception was measured across each plot on 11 occasions over the 2012-13 season, beginning shortly after budbreak until the end of leaf-fall. The methodology followed that of Palmer et al. (2002) using nine quantum sensors (Palmer, 1987) equally spaced along a horizontal bar 25 cm above ground level which was mounted on a four-wheeled trolley. The trolley also carried a data logger (CR10, Campbell Scientific Inc., Logan, USA) and a 12-V DC power source. The nine sensors measured below-canopy irradiance, while a tenth sensor mounted on a telescopic pole measured incoming irradiance above the canopy. The data logger was programmed to record instantaneous readings from every sensor at 0.25 s intervals when a switch on the handle of the trolley was depressed. The trolley was pushed at walking speed down the row and the switch was depressed adjacent to the first tree trunk of each plot and released adjacent to the third tree trunk of that plot. The process was repeated on the other side of the row, which achieved a total of 15-24 scans per plot. On each occasion, readings from all plots were complete within one hour. Before and after each data set was collected, open sky readings were taken for all sensors by recording 10 seconds of data in the orchard roadway. This allowed the determination of calibration coefficients for each below-canopy sensor to the above-canopy sensor. Canopy light interception was calculated as the difference in mean irradiance above and below the canopy divided by the mean irradiance above the canopy. To avoid the effect of differing sun angles, measurements were made under totally diffuse light conditions.

Shortly before harvest, total fruit numbers were recorded on the centre tree of each nine-tree plot. Within one day of the first commercial pick, 40 fruit were sampled at random from each centre tree for assessment of fruit weight. Tree mean values for each variate were subjected to ANOVA procedures

in Genstat v14 (VSN International Ltd, UK) using a significance of $\alpha \leq 0.05$, and means separations were conducted using least significant differences (LSDs) at 5%. Residual plots showed that all data were approximately normally distributed.

Results and Discussion

Total BCA after pruning in winter 2012 averaged 66.9 cm² and did not differ among treatments.

Hydrogen cyanamide applied as a dormancy breaker over the whole orchard on 23 August 2012, resulted in budbreak on 3 September. Full bloom was estimated to be at 27 September.

In ASE treatments, recorded spur + terminal (S&T) FBD did not differ from target FBDs set prior to budbreak (Table 1). S&T FBDs in ASE trees were about one half (49%, ASE6) to one fifth (21%, ASE2) of those on unmodified trees. In ASE treatments, lateral buds on one-year-old shoots were counted at flowering, but removed immediately afterwards. Consequently, total FBDs in ASE treatments were only 17% (ASE2 2.9 vs Unmod2 16.9 buds.cm⁻²) to 41% (ASE6, 5.5 vs Unmod6 13.5 buds.cm⁻²) of those on unmodified trees (Table 1).

Overall, ASE4 and Unmod4 treatments carried 289 fruit.tree⁻¹ at harvest, close to the commercial target of 275 fruit.tree⁻¹. At-harvest crop loads (number of fruit harvested.cm⁻² BCA after pruning) did not differ from target crop loads apart from in ASE6, which achieved a harvest crop load of 4.5, significantly ($P=0.05$) lower than 6 (but not lower than Unmod6 (5.2)) (Table 1). Within each target crop load (2x2, 1x4, or 1x6 fruit.cm⁻² BCA), harvest crop loads did not differ between ASE and Unmodified treatments; however, the trend in all cases was for harvest crop loads on ASE trees to be slightly less than those on unmodified trees.

Mean fruit weight (MFW) varied from 156 g to 186 g. Comparison of the main effect 'ASE versus Unmod', using harvest crop load as a covariate, showed that ASE treatments produced fruit that were on average 17.4 g heavier than those on unmodified trees (Table 1). The main effect of 'target bud density' on MFW was not significant ($P=0.061$) when harvest crop load was used as a covariate.

ASE6 trees produced a calculated yield of 129 t.ha⁻¹, significantly greater than that of ASE2 trees (96 t.ha⁻¹), but neither of these yields differed statistically from those in other treatments (112-122 t.ha⁻¹) (Table 1). Over the season, proportional light interception by the tree canopies followed a curve typical of that reported in previous studies (Palmer and Jackson, 1977; Palmer, 1988; Palmer et al., 2002)(Fig. 1). As the canopies developed in spring, light interception rose rapidly until early summer

(November) and then remained constant for most of the growing season until a decline corresponding with leaf-fall commenced in autumn (first week of April). In unmodified treatments, which did not differ in light interception from each other at any time during the season, light interception increased from an initial value of ~29% 2-3 weeks after budbreak (WABB), to a maximum of 59-62% by 8-11 WABB. It was expected that light interception would not differ among unmodified treatments, as all treatments were unthinned prior to hand thinning at the end of November and the crop loads set at this time were all moderate to heavy, reducing the tendency of trees to produce a second flush after the seasonal termination of shoot growth in mid-December. In a cultivar x spacing trial in New Zealand, with tree densities of 1190 to 2198 trees.ha⁻¹ and tree height of 2.1 m, Palmer et al. (2002) found mid-season light interception values of up to ~55%. The higher mid-season light interception (59-62%) recorded here may be explained by the greater tree density (2353 trees.ha⁻¹) and tree height (3.5 m).

Table 1. Mean spring spur + terminal (S&T) and total (Tot) floral bud density (FBD), at-harvest crop load, mean fruit weight and calculated yield from 'Royal Gala' apple trees either with artificial spur extinction (ASE) or unmodified (Unmod). In ASE, target floral (fl) and, as a result, fruiting (fr) bud densities, were set before flowering. Target fruiting bud densities were set at hand thinning in Unmod. Target crop loads on both ASE and Unmod were set at hand thinning. In ASE treatments, flower clusters on lateral buds of one-year-old shoots were removed immediately after counting, reducing Tot FBD from the numbers in brackets to S&T FBD.

Thinning	Target fl/fr bud density (buds.cm ⁻² BCA)	Target crop load (fruit.cm ⁻² BCA)	S&T FBD (buds.cm ⁻² BCA)	Tot FBD (buds.cm ⁻² BCA)	Harvest crop load (fruit.cm ⁻² BCA)	Mean fruit weight (g) ¹	Yield (t.ha ⁻¹)
ASE	2	2x2	2.9	2.9 (9.2)	3.3	184.4	96
	4	1x4	4.5	4.5 (11.0)	3.8	186.0	113
	6	1x6	5.5	5.5 (10.9)	4.5	170.9	129
		mean	4.3	4.3 (10.3)	3.9	180.4	113
Unmod	2	2x2	14.0	16.9	4.3	169.0	112
	4	1x4	13.2	15.3	4.9	163.5	122
	6	1x6	11.3	13.5	5.2	156.4	118
		mean	12.8	15.2	4.8	163.0	117
Thin	P-value		<0.001	<0.001	0.004	0.007	0.608
	LSD (5%)		0.91	1.43	0.61	9.94	18.0
ThinxBud	P-value		<0.001	0.018	0.758	0.645	0.438
	LSD (5%)		1.58	2.47	1.06	15.05	31.2

¹ Harvest crop load used as covariate ($P=0.443$).

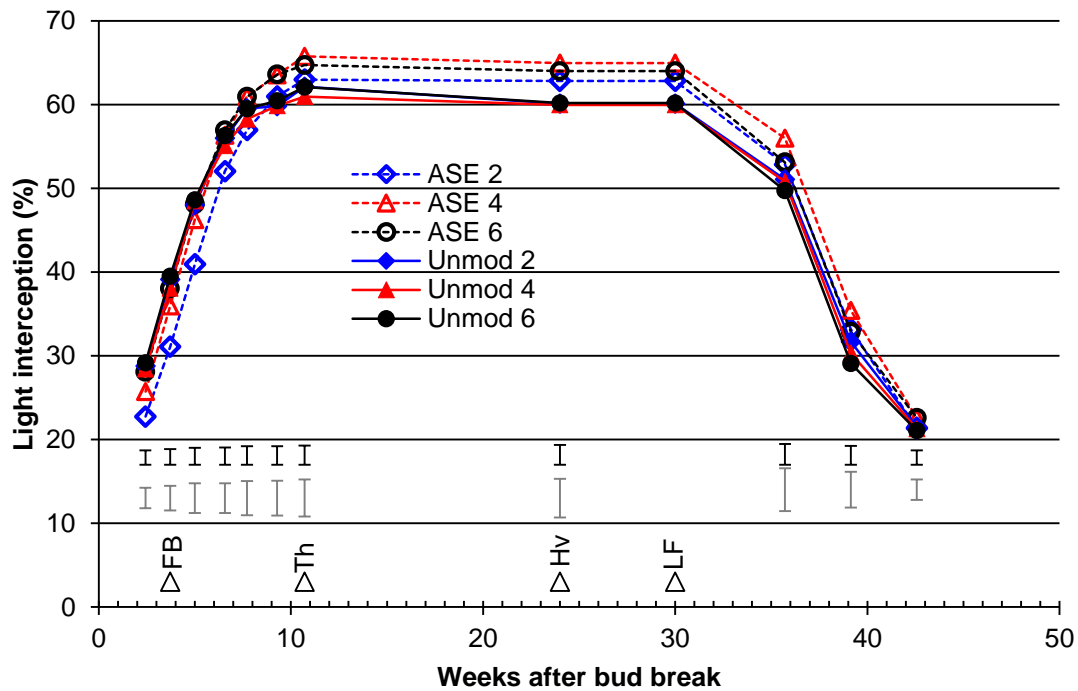


Figure 1. Mean light interception by 'Royal Gala' apple trees on 11 occasions through the 2012-13 season from shortly after budbreak to final leaf fall. Trees were either treated with artificial spur extinction (ASE) or unmodified (Unmod) at three target floral and fruiting bud densities set either before flowering in ASE or at hand thinning in Unmod. Top, black error bars represent LSD (5%) of ASE v. Unmod main effect. Bottom, grey error bars represent LSD (5%) of ASE/Unmod x bud density interaction. Timing of full bloom (FB), final hand thinning (Th), harvest (Hv) and the beginning of leaf fall (LF) are shown.

Despite removing S&T buds in ASE6 trees to achieve a FBD of less than half of that in unmodified trees, early-season light interception (prior to 8 WABB) by ASE6 trees did not differ from that by unmodified trees (Fig. 1). In ASE4 trees which had only about one third of the S&T buds of unmodified trees, there was a small (2 units) and short-lived (up to 4 WABB) reduction in light interception compared with unmodified and ASE6 trees. In ASE2 trees this trend continued, with light interception reduced by 5-7 units persisting until 7 WABB. In unmodified trees, the rate of increase in light interception slowed markedly from 8 WABB (Fig. 1). In comparison, in ASE trees the increase in light interception slowed less abruptly, so that the maximum light interception by ASE trees (63-65%) was higher than by the unmodified trees (59-62%).

These results are comparable to those of light interception modelling in 3-year-old 'Galaxy' grown as Solaxe trees with and without ASE applied during centrifugal training (Willaume et al., 2004). That study showed that centrifugal training significantly increased whole-tree light interception using a single measurement after the first growth arrest.

Interestingly there was no difference in light interception between unmodified and ASE6 trees before 8 WABB, despite floral bud densities in the ASE treatment having reduced by more than half. This suggests that the leaf areas of the bourses and bourse shoots in ASE6 trees were greater than in unmodified trees and thus compensated for fewer buds than in unmodified trees. The leaf areas of both bourse and bourse shoots have been shown to be greater when originating from terminal buds of short to medium length (2.5-30 cm) shoots than those from spur structures (Tustin et al., 2011b) and the proportion of this category of shoots is known to be greater in ASE trees than in unmodified trees (van Hooijdonk et al., 2010; Tustin et al., 2011a). Removal of competitive shoots and the increased sink effect of the whole fruiting shoot have been suggested as reasons for increased leaf area of fruiting bourses and associated bourse shoots in centrifugally trained trees (Willaume et al., 2004). Reduction in competition between fruit and other sinks by early flower and fruitlet thinning has also been shown to increase the number of shoots (primarily bourse shoots) in apple trees (Quinlan and Preston, 1968). Very early removal of competitive fruit clusters through ASE has been shown to improve fruit set, a response thought to be a consequence of increased resource supply to the remaining floral sinks (Tustin et al., 2011a; Breen et al., 2014). Consequently, both greater leaf area and the greater proportion of bourse shoots in ASE treatments are probably the consequences of reduced early-season sink competition.

Differences in the rate of increase in light interception approaching maximum light interception may also reflect a higher proportion of bourse shoots on ASE trees than on unmodified trees, as shoots terminate later than spurs (Palmer and Jackson, 1977). Higher maximum light interception by ASE trees may be the consequence of the increased proportion of shoots (which have greater leaf area) and fewer spurs on ASE trees. Lower early-season light interception in ASE4 and ASE2 trees is likely to be a reflection of reduced leaf area as a result of lower bud density. The pattern of light interception of ASE2 over the whole season appears lower than that in other ASE treatments. It is possible that at bud densities of 2 buds.cm⁻² BCA and less, the remaining buds do not produce

sufficient leaf area to intercept as much light as at higher bud densities. However, these data show that this response only occurs at bud densities well below that required for commercial production. Comparing mid-season light interception versus fruit yield relationships presented in Palmer et al. (2002) with ASE light interception of 63 to 65% measured in this work, predicted a yield of 94 to 97 t.ha⁻¹ for 'Royal Gala' (Yield = 1.49 x light interception). This was very much lower than the yields recorded from 'Royal Gala' in the current study. Recorded yields were more similar to the 118 to 122 t.ha⁻¹ predicted to be achieved for 'Braeburn' and 'Fuji' response curves of Palmer et al. (2002) (Yield = 1.88 x light interception). This response cannot be explained in terms of the increased MFW or light interception in ASE, as yield from unmodified trees also follows the 'Braeburn'/'Fuji' relationship. However, at least part of the ~11 t.ha⁻¹ increase in yield in ASE4 and ASE6 over the unmodified 4 and 6 treatments may be attributed to the 3-4% higher mid-season light interception in ASE4 and ASE6 which, using this relationship, predicts an increase of 5.6-7.5 t.ha⁻¹. Yield is determined by the intercepted total radiation over the whole season, so care must be exercised in simple comparisons of this kind. Mid-season light interception is a 'snapshot' of a current situation and does not account for factors such as season length and radiation environment, which alter total seasonal radiation receipt and are likely to differ among different studies.

Conclusions

Fruit yield of apple orchards is related to total seasonal light interception by the orchard canopy. ASE treatments aimed at achieving medium to high commercial crop loads (ASE4 and ASE6) removed more than half and up to two thirds of S&T floral buds and their associated leaf area compared with those of unmodified trees. Despite this, light interception was not reduced in the ASE6 treatment, and in ASE4 treatment, reduction in light interception was both small (2-2.4 units) and short lived, occurring before petal fall. More intensive removal of floral buds in the ASE2 treatment further reduced light interception (5-7 units) and increased the duration of the effect, until about two weeks after petal fall. Despite early season reductions in light interception, for most of the season light interception in the ASE treatments was higher than in unmodified treatments and will have contributed to the increased yield in ASE treatments.

ASE trees produce a higher proportion of short- to medium-length bourse shoots compared with unmodified trees (van Hooijdonk et al., 2010). The greater leaf area of bourse shoots compared with

spurs increases tree leaf area, leading to increases in light interception by leaves in close proximity to developing fruit, and increased yield potential in the current season. Increasing the population of terminal buds may also increase the fruit size potential for fruit in the following season, as these buds are known to produce larger fruit than those on spurs (Tustin et al., 2011b).

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Chapter 5. A re-evaluation of the role of carbohydrate reserves in fruit set and early season growth of apple

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Abstract

In apple (*Malus x domestica* Borkh.), carbohydrate, nitrogenous, and mineral reserves are considered essential for maintaining tree functions and root growth during dormancy, and supporting early spring growth before trees become autotrophic. To improve our understanding of the mechanisms regulating fruit set in apple, we require a greater understanding of the role of these reserves and their contribution to early-season tree development. In high yielding 'Royal Gala' trees, we used artificial spur extinction (ASE) to remove whole buds in late dormancy and reduce floral and vegetative sink numbers compared with post fruit drop hand thinning (PFD). On trees of both of these thinning treatments, early and late (natural) post-harvest defoliation treatments were used to manipulate post-harvest carbohydrate reserve replenishment and alter carbohydrate reserve concentration through winter and in the following spring. This allowed us to investigate the interaction of total early season sink demand and altered carbohydrate reserve supply on fruit set. ASE is a crop load management tool that uses hand-thinning of whole buds in late dormancy to set targeted floral bud densities consistently on every limb. Early defoliation manually removed all leaves on treated trees 12 days after final commercial harvest. Natural autumn defoliation was protracted, with visibly healthy leaves

still present on trees 2.5 months after application of the early defoliation treatment. Thinning treatment had no effect on winter carbohydrate concentration. In the roots of trees where natural defoliation occurred, 32% of total non-structural carbohydrate present in winter was metabolised prior to full bloom and thereafter concentration remained constant. Early defoliation reduced winter total non-structural carbohydrate concentration in root tissue by 45% and the starch component of this by 51% compared with natural defoliation. In trees defoliated early, total non-structural carbohydrate concentration and starch concentration in roots did not decline at any stage from dormancy up to nine weeks after full bloom. Early defoliation also reduced winter total non-structural carbohydrate concentration in shoots by 10% and winter starch concentration in shoots and spurs by 37 to 60%. In shoots and spurs, the concentration of total non-structural carbohydrate and starch declined between dormancy and two weeks after full bloom, after which there was no further attenuation. Fruit set did not differ between defoliation treatments despite large differences in total non-structural carbohydrate and starch concentration prior to bud break. Any decline in the concentration of stored carbohydrates occurred before the period 2 – 4 weeks after bloom, in which competition among sinks for a limited carbohydrate source is thought to affect fruit set. Therefore, differences in carbohydrate reserve concentration at resumption of growth in spring did not appear to have any direct effect on fruit set. Concentration of sorbitol in the spur and root and glucose + fructose in the spur increased as the leaf canopy developed from one week before full bloom to two weeks after full bloom, before the fruit set sensitive period 2 – 4 weeks after bloom. Consequently, factors affecting the supply of newly synthesised photosynthates, such as early season light interception and leaf area may play a greater role in carbon supply for fruit set and initial fruit development than carbohydrate reserves. Assessment of shoot composition at the end of the season showed that a greater proportion of shoots (as opposed to spurs) was present in trees where both winter carbohydrate concentration was high (natural defoliation) and early season sink number was reduced (ASE). Thus stored carbohydrates appear likely to be important in early development of vegetative sinks, but do not contribute directly to fruit set.

Keywords

bud break, flowering, artificial spur extinction, defoliation, root, stem, spur, starch, sorbitol

Introduction

In deciduous fruit trees such as apple (*Malus x domestica* Borkh.), carbohydrate reserves are considered essential for maintaining tree functions (respiration, cell differentiation, bud development, phloem transport) and root growth during dormancy (Priestley, 1964a), and supporting early spring growth, before trees become autotrophic (Oliveira and Priestley, 1988; Tromp, 2005). Carbohydrates form the greatest portion of the reserves, and although nitrogenous compounds and minerals are also stored by the tree, and their availability as well as that of root produced hormones may affect physiological aspects such as early shoot growth (Hennerty and Forshey, 1972; Tromp, 1983; Faby and Naumann, 1986; Webster, 2005), they are not reported on in this paper.

Starch is the primary reserve carbohydrate in apple trees. In apple and pear, the sugar alcohol sorbitol is the major end product of photosynthesis, the dominant form of carbohydrate transported, and is an important soluble reserve. Sorbitol is converted into other sugars such as sucrose, glucose and fructose, as well as starch (Kandiah, 1979a; Tromp, 1983; Oliveira and Priestley, 1988). Other soluble carbohydrates such as galactose, raffinose, stachyose and *myo*-inositol are also found (Archbold et al., 2011). As carbohydrates are readily interconverted, when considering carbohydrate reserve resource, the concentration of individual carbohydrates is probably less important than the total concentration of all non-structural carbohydrates (Tromp, 1983).

Apple trees are not regarded as having specialist storage structures because most structures show similar fluctuations in available carbohydrate through the season (Priestley, 1964b). Reserves from all structures contribute to spring growth. A large proportion of assimilate is moved to the root in autumn where it is converted to starch, and this resource is metabolised over winter and in the following spring (Priestley, 1964b; Hansen, 1967; Kandiah, 1979b). In absolute terms, bark is less important as a store than roots, however bark and wood also act as sites of carbohydrate storage and remobilisation, even in wood more than 20 years old (Priestley, 1964b; Hansen and Grauslund, 1973). One year old shoots appear to act more as a conduit for carbohydrates rather than a site of storage (McQueen et al., 2004b; McQueen and Minchin, 2005).

In young trees, 18 - 50% of the dry weight of the perennial structure and 50 - 60% of the root dry weight may be composed of extractable carbohydrates (Priestley, 1964a, 1970; Kandiah, 1979a). Most authors report a large and rapid decline in extractable carbohydrate content of most structures from close to or before bud break, up to a period around two to four weeks after bud break. In young

trees and rootstocks, starch and soluble carbohydrates in the roots, shoot and trunk may decline by 50 - 60% over this period followed by a rise shortly after (Priestley, 1963; Hansen and Grauslund, 1973; Kandiah, 1979b). In young rootstocks, 20 - 25% of root and bark carbohydrates move to new shoots in spring, but 28 - 50% remain in the root for periods of more than one season (Hansen, 1967; Kandiah, 1979b). The remobilisation of these carbohydrate reserves to sites of active growth in spring supports the hypothesis that trees are dependent on them in the early season. However, the reliance on these reserves is thought to be short-lived, only lasting to around bloom, as production of newly synthesised carbohydrates starts very early in spring and increases rapidly as the new leaf canopy develops (Hansen and Grauslund, 1973; Lakso et al., 2006b). Newly fixed ^{14}C from opening buds prior to flowering (just past green tip to pink) is subsequently found in leaves and fruit (Quinlan, 1969; Hansen, 1971). Very shortly after flowering (petal fall / one week after bloom) significant portions of ^{14}C applied to primary and spur leaves are recovered in developing fruit (Tustin and Lai, 1990; Corelli Grappadelli et al., 1994) and the net carbon exchange of the tree becomes increasingly positive (Heinicke and Childers, 1937). The sorbitol content of shoot xylem sap is sufficient to support initial fruit set until one week after bloom, however, increasing demand from other active meristems, particularly rapidly growing shoots, is thought to result in a carbohydrate deficit around two to four weeks after full bloom, and by two weeks after bloom, sorbitol content of shoot xylem sap is probably no longer adequate to supply fruit growth requirements (Lakso et al., 1998; Lakso et al., 1999; Archbold et al., 2011). This slows fruit growth and induces abscission of the slowest growing fruit (Greene et al., 2005; Lakso et al., 2006a). Fruit set is substantially reduced where early season photosynthetic capacity is reduced through shading or removal of spur leaves (Ferree and Palmer, 1982; Byers et al., 1991). Replenishment of reserves begins early, as soon as the first leaves expand (Priestley, 1960, 1963) and continues through the season until leaf abscission occurs in autumn (Oliveira and Priestley, 1988). A second period of low carbohydrate content may occur, coinciding with the period of greatest shoot extension (Oliveira and Priestley, 1988).

The exact role of reserve carbohydrates and the degree of their contribution to annual seasonal growth are not clear (McQueen et al., 2005; Tromp, 2005; Archbold et al., 2011). Large proportions of stored carbohydrates may be mobilised in early spring, and they may form an important part of the early (prior to bloom) building materials in spurs and shoots, but the majority are thought to be respired during early season metabolism, as only 13 - 17% become part of the permanent structure of

the tree (Hansen, 1967, 1971; Hansen and Grauslund, 1973). In mature 'Golden Delicious' trees, fruit set was not correlated with spur or shoot carbohydrate concentration measured at stages between dormancy and three weeks after full bloom (Hennerty and Forshey, 1971, 1972). In the same cultivar, early defoliation (1 – 2 months before natural defoliation) greatly reduced starch content of one year old shoots between dormancy and flowering the following season, and significantly reduced fruit set, even though starch and soluble carbohydrate content of flower clusters did not differ (Faby and Naumann, 1986).

Some studies have shown a positive relationship between reserve carbohydrate concentration and new growth on spurs (Hennerty and Forshey, 1972) or vegetative growth in nursery trees (Abusrewil and Larsen, 1981; Abusrewil et al., 1983), but Priestly found no relationship to growth in young apple rootstocks (Priestley, 1963, 1964a).

Much of the current understanding of the role of carbohydrate reserves in apple is based on research conducted on young trees or rootstocks situated in the northern hemisphere. In New Zealand, there is a long period between harvest and natural defoliation which gives ideal conditions for replenishment of reserves after harvest and before dormancy (Tustin et al., 1997; Wünsche and Palmer, 1997). A number of authors have successfully used leaf removal shortly after harvest as a method to reduce replenishment of carbohydrate reserves prior to winter and produce differences in carbohydrate concentration among treatments during dormancy and in the following spring (Abusrewil and Larsen, 1981; Faby and Naumann, 1986). In research presented here, we used early (12 days after harvest) and natural defoliation (healthy leaves were still visible on trees 2.5 months after harvest) to alter the carbohydrate reserve status of mature 'Royal Gala' trees in a commercial orchard in New Zealand.

Vegetative and fruit growth responses are closely related to the earliness and degree with which the reproductive sink demand is reduced (Quinlan and Preston, 1968; McArtney et al., 1996; Byers, 2003; Embree et al., 2007). It is thought that through removal of competing sinks very early, competition for limited resource supply among remaining sinks in the early season is reduced, thereby improving fruit set on retained buds (Lauri and Terouanne, 1999; Tustin et al., 2012). Artificial spur extinction (ASE) is a thinning method in which buds are manually removed between late dormancy and early bud break in order to manage the distribution and reduce the density of floral buds through the whole canopy. Final fruit densities are set by hand after fruit drop is complete (Lauri and Lespinasse, 1999,

2000; Lauri et al., 2004; Tustin et al., 2012). We compared trees managed using ASE with trees where bud numbers were not modified, but final fruit densities were set by post fruit drop hand thinning (PFD). Combining these thinning methods with defoliation treatments, allowed us to examine the interaction of sink demand and carbohydrate reserve concentration on fruit set. We hypothesised that reducing the concentration of carbohydrate reserves through early defoliation would increase carbohydrate limitation during flowering and early fruit development the following spring, and result in reduced fruit set. We also hypothesised that by reducing total sink demand through reducing the density of competing sinks using ASE, within-bud fruit set would be increased compared with PFD.

Materials and Methods

This study was conducted on a high yielding commercial 'Royal Gala'/'M9' orchard, planted in 2005 at 3.4 x 1.25 m spacing and situated in Hawke's Bay, New Zealand. Trees had been pruned to a Central Leader Tall Spindle system since planting and were managed under conventional spray and irrigation practices with a target of 275 fruit.tree⁻¹ and 100 t.ha⁻¹. This orchard block, including experimental trees, received an annual dormancy-breaking spray in order to advance flowering date and compress the flowering period. In winter 2011, trees of similar size were selected to form five blocks each with two plots of three adjacent trees down a single row. Within each block, each plot of three trees was randomly allocated to a single thinning treatment, either artificial spur extinction (ASE) or post fruit drop hand-thinning (PFD). Each winter these trees were pruned to standardise the number of limbs to six to seven limbs per metre of effective canopy height. Very little adjustment was required after the first winter. Where necessary, remaining limbs were tied down to 10 – 15° below the horizontal to reduce vigour. ASE and PFD treatments were imposed following the methods of Breen et al. (2015) at bud densities of 6 buds.cm⁻² basal branch cross-sectional area (BCA). These trees formed part of a larger study and consequently each plot had the same treatment applied to it annually for three seasons (2011 - 12, 2012 - 13 and 2013 - 14). The sum of individual BCAs for each tree showed that a crop load of about 4 to 5 fruit.cm⁻² BCA would achieve approximately the same number of fruit per tree as the commercial target for the orchard. No chemical thinners were applied to any treatment. All treatments were hand thinned to target crop loads in November, after final fruit drop was complete. Crop loads were set at a branch unit level setting a heavy crop load of 6 fruit.cm⁻² BCA with a single

fruit per bud. In a small number of cases two fruit per bud were set to compensate for floral buds that failed to set fruit. Fruit on lateral buds of one-year-old shoots were removed.

After harvest in February 2013, in each plot, the centre tree and the tree most similar in size and form to it were selected to examine the role of carbohydrate reserves in flowering and fruit set the following season. The third tree in each plot was not used. The centre tree formed part of ongoing studies and was therefore left to undergo natural autumn leaf abscission (natural defoliation). On the other tree in each plot, every leaf was removed by cutting through the petioles on 11 March, 12 days after final harvest (early defoliation).

During the following dormant period (winter 2013), three representative, mid-canopy limbs were selected and tagged on all treatment trees. One of these limbs was used to record phenological development early in the following spring and all three were used to record subsequent individual limb floral bud density (FBD, number of floral buds.cm⁻² BCA) and fruit set. Floral bud numbers on each limb were converted to spur + terminal (S+T) and total (S+T + lateral) floral bud density using BCA.

Fruit set was recorded after natural fruit drop was complete and prior to setting final crop loads.

Within-bud fruit set patterns were analysed by recording the number of floral S+T buds setting each of seven classes of fruit set (0 – 6 fruit per bud). This was converted to their proportion of the total floral bud number for that limb. Fruit set density (number of fruit.cm⁻² BCA) and mean fruit set per floral bud was calculated for each limb. The calculated mean of flowering and fruit set data from the three limbs per tree provided an estimated tree mean. Before pruning the following winter (2014), two of these limbs were used for investigation of the population of annual shoots produced. On each limb the number of spurs (shoot length ≤ 2.5cm with compressed internodes) and shoots (> 2.5cm) produced in the previous season was recorded. The numbers of spurs and shoots were then converted to spur, and shoot densities using BCA recorded at the same time.

Root, shoot and spur tissue samples were collected from all trees beginning at dormancy in winter 2013 and continuing until after final fruit drop was complete at the end of November 2013. Sampling dates were 8 August (dormant), 20 September (1 week before full bloom, about 3.5 weeks after bud break), 11 October (2 weeks after full bloom), 1 November (5 weeks after full bloom) and 29 November (9 weeks after full bloom). Samples collected in the field were immediately placed on dry ice until freeze drying within four hours. For root samples, the area under each tree was divided into 10 sectors of about 36°, radiating from the trunk. At each sampling date two opposing sectors were

used for sampling. Only live roots of 2 – 5 mm diameter, within 200 mm of the soil surface and 1m of the trunk, were collected, ensuring that they originated from the tree of interest. Roots were washed before freeze drying. Low numbers of one-year-old, 12 – 30 cm long shoots present on these trees limited sampling to five shoots per tree and only on the first, third and last date. The middle 50% of each shoot was retained and the remainder discarded. Ten spurs were collected from random positions within the mid-canopy of each tree on all five dates. Each spur consisted of a lateral spur shoot produced in 2011 - 12, some compressed internodes where bracts and basal spur leaves were present in spring 2012, all of 2012 - 13 season spur growth and all of 2013 - 14 season spur growth. From the second sampling date onwards, only shoots and spurs that were flowering or fruiting were collected. Leaves, peduncles, fruit and flowers were not included because our primary interest was in observing changes in carbohydrate concentration of structures where reserve carbohydrates had been held. After freeze drying, samples were stored at -20°C until grinding using an IKA A11b Analytical mill in which the samples were kept frozen using liquid Nitrogen. Approximately 0.05 g (weighed to 4 decimal places) of dried, ground tissue was extracted in 80% ethanol at 60°C for 1 h, with the addition of adonitol (Sigma–Aldrich, A5502, New Zealand) as the internal standard. Extracted samples were centrifuged and the supernatant decanted. The residue was re-suspended in 80% ethanol, re-spun and the supernatants combined. The insoluble residue was transferred into an Erlenmeyer flask and analysed for starch as per Smith et al. (1992). A sub-sample of the supernatant was taken and the ethanol was blown off using a stream of nitrogen gas and then samples were re-dissolved in ultra pure water. The sugars were analysed using DIONEX ICS-3000 Reagent-Free™ IC (RFIC™) system with a CarboPac MA1 column. Concentrations of starch, and each detected soluble carbohydrate were expressed in mg.g⁻¹ of tissue dry weight (dw). Within each tissue type, the concentration of total non-structural carbohydrates (starch + soluble carbohydrates) and total soluble carbohydrates were investigated by summing their components. Of the soluble carbohydrates, glucose and fructose were found in low concentration (<3.4 mg.g⁻¹dw) and in similar concentration at each sampling date, and a similar situation occurred with raffinose and stachyose. Consequently, glucose was grouped with fructose, and raffinose with stachyose for statistical analysis. *myo*-Inositol, galactose and 10 unidentified soluble carbohydrates were periodically recorded at concentrations up to 7.2 mg.g⁻¹dw. However, as their concentrations were generally in the range 0 to 1 mg.g⁻¹dw they were also summed for statistical analysis.

Immediately prior to the first commercial harvest, the number of fruit on each tree was counted and a random sample of 50 mature fruit per tree were picked for calculation of mean fruit weight and yield. For flowering, fruit set, shoot population, and mean fruit weight, plot mean values were subjected to ANOVA procedures in Genstat v17 (VSN International Ltd., UK) using a significance of $P \leq 0.05$. For analysis of carbohydrate concentration, where a series of samples were taken from the same trees over time, the repeated measures function in Genstat v17 ANOVA procedures was used. In all cases, means separations were conducted using least significant differences at 5%. Distribution of residual values appeared normal in all circumstances.

Results

Fruit yield calculated from individual ASE and PFD trees in February 2013, prior to leaf removal, was high, averaging 123 t.ha^{-1} , with no difference among treatments. Natural defoliation commenced on about 1 April, but visibly healthy leaves were still present on trees until the latter half of May, more than two months after application of the early defoliation treatment, and the last leaves only abscised in mid-June. The dormancy breaking chemical Erga® was applied to the whole orchard on 19 July 2013 and resulted in bud break of 59% of spur and terminal buds by 26 August. Neither thinning ($P=0.349$) nor defoliation ($P=0.613$) treatments affected the proportion of buds that were at bud break on this date. Estimated full bloom occurred on 27 September, however flower development in trees defoliated early was slightly delayed, with a lower proportion of flower clusters at open cluster stage (15%) on 16 September compared with naturally defoliated trees (55%, $P=0.028$). Thinning treatment had no effect on the proportion of flower clusters at open cluster stage on this date ($P=0.437$) and there was no thinning x defoliation interaction.

Setting a spur + terminal bud density of 6 buds per cm^2 BCA on ASE trees resulted in a spur + terminal floral bud density of 5.9 buds per cm^2 BCA, about half of that in PFD trees (Table 1).

Although lateral floral bud density was higher in ASE trees than PFD trees, lateral floral buds in ASE trees were removed at flowering. Early defoliation had no effect on floral bud density the following spring ($P=0.653$ to 0.737) and there were no thinning x defoliation interactions ($P=0.456$ to 0.746).

Compared with PFD, ASE reduced spur + terminal fruit set on a whole-limb basis (fruit set density) by 25% but increased within-bud fruit set (mean number of fruit set per bud) by ~38% from 0.8 to 1.1 (Table 1). Increased within-bud fruit set was caused by about 50% fewer floral buds setting zero fruit

and greater proportions setting two and three fruit per bud (Table 2). Early defoliation had no effect on fruit set density ($P=0.169$) or within-bud fruit set ($P=0.208$) and there was no thinning x defoliation interaction ($P=0.142$ to 0.349).

Table 1. Flowering and fruit set on 'Royal Gala' trees in New Zealand in 2013 thinned using artificial spur extinction (ASE) or post fruit-drop hand thinning (PFD) and either allowed to undergo natural autumn defoliation or receiving early manual removal of leaves, 12 days after harvest. Floral bud density (FBD, number of floral buds.cm⁻² branch basal cross-sectional area (BCA)) on spur + terminal (S+T) and lateral buds on one-year-old shoots (lateral) were recorded at flowering, while fruit set density (FSD, number of fruit.cm⁻²BCA) and mean fruit number per floral bud (MFB) were recorded on S+T sites after natural fruit drop. Lateral floral buds on ASE trees were removed after counting.

		S+T FBD	Lateral FBD	S+T FSD	S+T MFB
Thinning	ASE	5.9	1.8	6.6	1.1
	PFD	11.1	0.5	8.8	0.8
	<i>P</i> -value	<0.001	0.001	0.005	<0.001
Defoliation	Natural	8.6	1.2	8.2	1.0
	Early	8.4	1.1	7.2	0.9
	<i>P</i> -value	0.653	0.737	0.169	0.208

Trees in this experiment had had the same thinning treatments applied to them for two seasons prior to defoliation. The thinning treatment applied over the previous seasons had no effect on the concentration of total non-structural carbohydrates or total soluble carbohydrates in any structure at any date during the carbohydrate sampling period. Apart from small ($4.6 - 4.7\text{mg.g}^{-1}\text{dw}$) differences in starch concentration in spurs at one week before full bloom (20 September (ASE > PFD)) and five weeks after full bloom (1 November (PFD > ASE)), thinning treatment had no effect on starch concentration in any structure on any date either (data not shown). There was no thinning x defoliation interaction effect on carbohydrate concentration. However, defoliation treatments did alter non-structural carbohydrate concentration.

Table 2. . The proportion of floral buds setting 0 to 6 fruit per bud after natural fruit drop and before hand-thinning on 'Royal Gala' trees in New Zealand in 2013. Trees were thinned using artificial spur extinction (ASE) or post fruit-drop hand thinning (PFD) and either allowed to undergo natural autumn defoliation or receiving early manual removal of leaves, 12 days after harvest. Data for buds setting 4, 5 and 6 fruit were not subjected to ANOVA because of high numbers of zeros.

		Number of fruit set per S+T bud						
		0	1	2	3	4	5	6
Thinning	ASE	12.8	41.1	37.0	8.1	0.9	0.1	0.0
	PFD	26.4	52.4	19.5	1.4	0.2	0.0	0.0
	<i>P</i> -value	0.025	0.165	0.004	<0.001			
Defoliation	Natural	20.2	44.6	29.1	5.1	0.9	0.1	0.0
	Early	19.1	48.9	27.3	4.4	0.1	0.0	0.0
	<i>P</i> -value	0.833	0.579	0.714	0.678			

Total non-structural carbohydrate concentration in roots was about 1.5 (early defoliation) to three (late defoliation) times greater than in shoots or spurs (Figure 1). In roots, starch comprised 79 - 90% of total non-structural carbohydrate, irrespective of defoliation treatment or sample time. Consequently, changes in starch concentration had a considerable effect on the concentration of total non-structural carbohydrates. In shoots and spurs, the starch component varied, but was much lower, never greater than 32% in spurs and 37% in shoots, and was generally less than 20% of the total non-structural carbohydrate present.

Sorbitol and sucrose comprised the largest fraction of soluble carbohydrates in all structures (Figure 1). In roots, sorbitol was found in fairly similar concentrations to sucrose, although sorbitol concentration increased when sucrose concentration decreased. In shoots and spurs sorbitol was present in much higher concentrations than sucrose. On some sampling dates, glucose + fructose were present in slightly higher concentrations in shoots and spurs than in roots, but generally concentrations of soluble carbohydrates other than sorbitol and sucrose did not differ widely among structures.

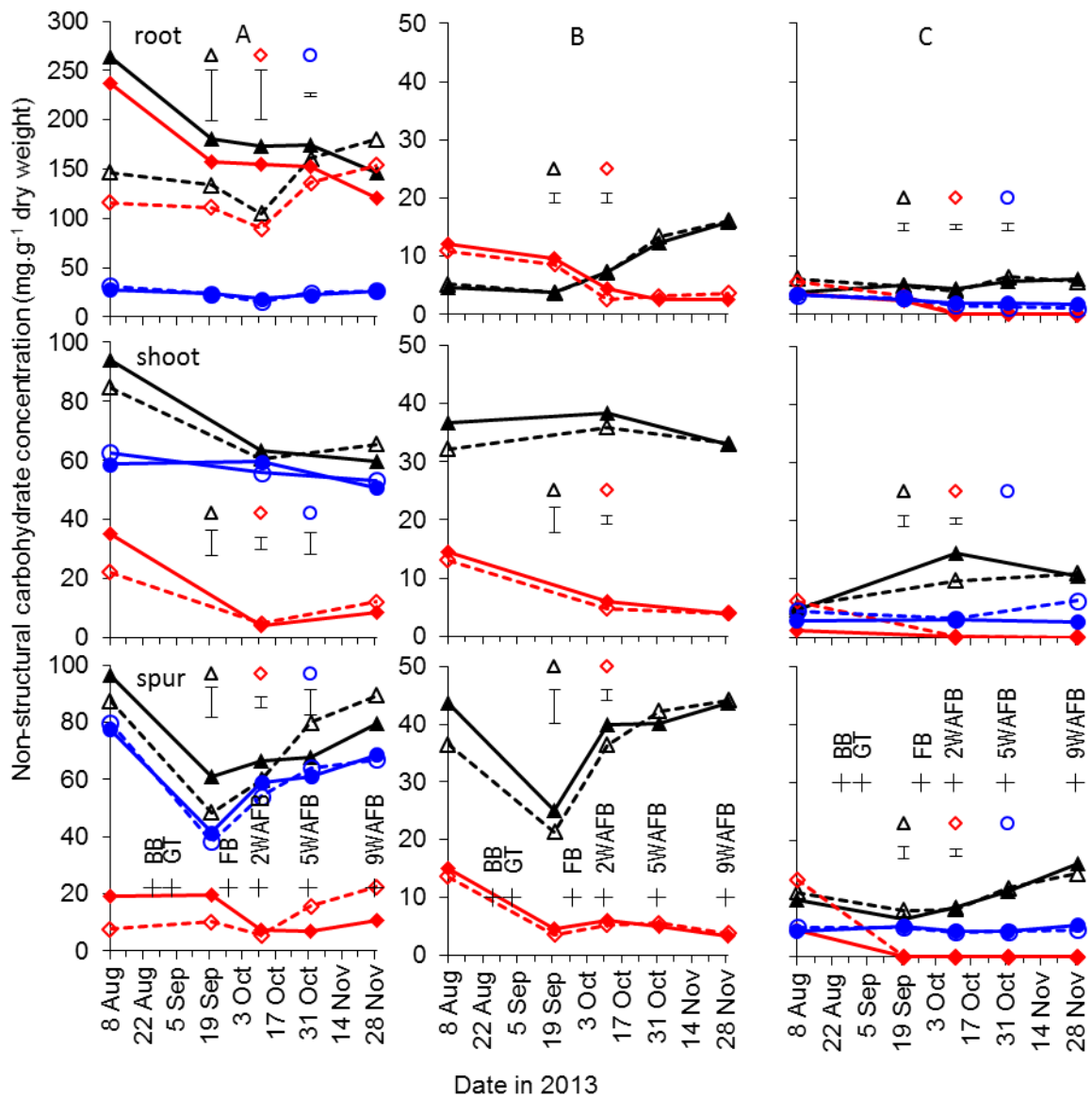


Figure 1. The concentration (mg.g^{-1} dry weight) of non-structural carbohydrates extracted from roots (top row), shoots (middle row) and spurs (bottom row) of 'Royal Gala' trees in New Zealand in 2013. Trees were either allowed to undergo natural autumn defoliation (solid lines) or were defoliated early, 12 days after harvest (broken lines). Lines in column A are total non-structural carbohydrates (black triangle), and its components starch (red diamond) and total soluble carbohydrates (blue circle). Columns B and C are soluble carbohydrates. Lines in column B are sorbitol (black triangle) and sucrose (red diamond); column C glucose + fructose (black triangle), raffinose + stachyose (red diamond) and myo-inositol + galactose + 10 unidentified carbohydrates (blue circle). The timing of bud break (BB), green tip (GT), full bloom (FB), 2-, 5- and 9-weeks after full bloom (WAFB) are shown for reference. Error bars with appropriate symbol for each carbohydrate are LSDs (5%) for defoliation

x date. Note that scale of Y axis in column A root (0 – 300) differs from shoot and spur (0 – 100), and differs from columns B and C (0 – 50).

In the roots of trees where defoliation occurred naturally, starch concentration declined by one third, from 237 to 157 mg.g⁻¹dw between dormancy and one week before full bloom, and thereafter did not change (Figure 1). Early defoliation had a considerable effect on winter root starch concentration, causing a 51% reduction compared with natural defoliation, to a concentration of 116 mg.g⁻¹dw. This concentration did not change over bud break, but increased later, between two and five weeks after full bloom, to similar concentrations as in the naturally defoliated trees. Changes in starch concentration in shoots and spurs over time followed a much reduced, but similar pattern to that of roots. In both shoots and spurs, early defoliation reduced winter starch concentration, but by two weeks after full bloom the greater decline in starch concentration in naturally defoliated trees resulted in a similar concentration between defoliation treatments. In spurs, the decline in starch concentration occurred from one week before full bloom until two weeks after full bloom. In shoots and spurs, starch concentration increased from two weeks after full bloom onwards, and in spurs of the early defoliation treatment, the increase was greater than in naturally defoliated trees.

The only effect that early defoliation had on sorbitol or sucrose concentration in any structure was to reduce spur sorbitol concentration during dormancy. In roots, in both natural and early defoliation, sorbitol concentration increased by about 300% from one week before full bloom until nine weeks after full bloom. Sucrose declined until two weeks after full bloom and especially over the bloom period. Shoot sorbitol concentration remained between 32 and 38 mg.g⁻¹dw during the whole sampling period. Spur sorbitol concentration was highly variable, declining by almost 50% from dormancy until one week before full bloom, and then undergoing a substantial increase to two weeks after full bloom. Similar to roots, in shoots, sucrose concentration declined after dormancy to a minimum level at two weeks after full bloom. In spurs, sucrose concentration declined prior to the bloom period, earlier than in roots, and thereafter remained constant until nine weeks after full bloom. Concentration of other soluble carbohydrates was generally low compared with sorbitol and sucrose and did not vary widely among sampling times. In roots, early defoliation resulted in greater concentration of glucose + fructose than natural defoliation; however this was not the case in shoots or spurs. In general, there was a trend for an increase in glucose + fructose concentration in all

structures by two to five weeks after full bloom. At dormancy raffinose + stachyose concentration in all structures was greater in trees defoliated early. Concentration of these sugars declined early in all treatments and all structures, and were completely depleted by one week before full bloom in spurs and two weeks after full bloom in roots and shoots.

Unfortunately, just prior to the planned first commercial harvest, the grower made an unannounced decision to harvest some fruit in the block. A small but unknown proportion of fruit was removed from some of the experimental trees in the process. This prevented exact calculation of yield (estimated to be ~126 t.ha⁻¹) and crop load. Assessment of mean fruit weight from remaining fruit revealed no differences in mean fruit weight between thinning ($P=0.070$) or defoliation treatments ($P=0.088$). Assessment of shoot composition prior to winter pruning 16 months after defoliation treatments were set up, showed that ASE reduced the density of spurs and spurs + shoots compared with PFD (Table 3). Early defoliation had no effect on the density of spurs or spurs + shoots. There was no interaction of main effects on spur or spur + shoot density. Analysis of the density of shoots showed an interaction between thinning and defoliation treatments (Table 4), with naturally defoliated trees treated with ASE producing a much higher shoot density than other treatments. The majority of these spurs and shoots had developed from lateral buds on the current season's bourse.

Table 3. The density (number per cm⁻² branch basal cross-sectional area) of spurs (shoot length \leq 2.5cm with compressed internodes) and spurs + shoots (shoot length > 2.5cm, Total) produced on 'Royal Gala' trees in New Zealand during the 2013-14 season and recorded in winter 2014. Trees had been thinned using artificial spur extinction (ASE) or post fruit-drop hand thinning (PFD) earlier in the season. Twelve days after harvest the previous season (16 months previously) trees were either allowed to undergo natural autumn leaf defoliation or received early manual removal of leaves 12 days after harvest.

		Spur	Total
Thinning	ASE	10.2	11.8
	PFD	15.9	16.4
	<i>P</i> -value	<0.001	0.005
Defoliation	Natural	11.9	13.6
	Early	14.2	14.6
	<i>P</i> -value	0.076	0.445

Table 4. The density (number per cm⁻² branch basal cross-sectional area) of shoots (length > 2.5cm) produced on 'Royal Gala' trees in New Zealand during the 2013-14 season and recorded in winter 2014. Trees had been thinned using artificial spur extinction (ASE) or post fruit-drop hand thinning (PFD) earlier in the season. Twelve days after harvest the previous season (16 months previously) trees were either allowed to undergo natural autumn leaf defoliation or were subject to early defoliation by manually removing every leaf.

Defoliation		Natural	Early
Thinning	ASE	2.5	0.6
	PFD	0.9	0.2
<i>P</i> -value <i>TxD</i>		0.026	
<i>LSD</i> (5%)		0.73	

Discussion

In this study, the concentrations of carbohydrates in roots, shoots and spurs, and the effect of early defoliation on these concentrations generally agree with data presented elsewhere (Hennerty and Forshey, 1972; Faby and Naumann, 1986; McQueen et al., 2004a). Differences in total non-structural carbohydrate and starch concentration between defoliation treatments, were observed in all structures. These differences, which reached 50% in some cases, illustrate the importance of the period between harvest and leaf fall to establishing carbohydrate reserves in high yielding trees. Although carbohydrate reserves in apple trees are not considered to be restricted to specific organs, starch accumulation occurs in greater concentration in the root both in very young trees (Hansen, 1967), and as this study shows, in mature trees. In results presented here, where natural defoliation occurred, winter reserve carbohydrate concentration, especially starch concentration, was high compared with early defoliation, and in the following season starch reserves were greatly reduced over bud break in roots, and over bloom in spurs. However, as McQueen et al. (2004a) have noted in shoots, and is confirmed in this research, a large portion of the starch reserve (66% in roots, 38% in spurs and 11% in shoots) may not be remobilised or metabolised during early spring. Where starch reserve concentration was reduced by 51% through early defoliation, starch reserves in the root did

not appear to be utilised at all, despite being part of the extractable pool and thus theoretically available for metabolism by the plant. Priestley (1970) found that young plants die before exhausting their apparently available carbohydrate supply. The reason why reserve carbohydrates are not more fully utilised by the plant despite there appearing to be sufficient sink demand is unclear, but may involve limitation of other critical resources such as nitrogen (Titus and Kang, 1982) or mineral reserves, or plant hormones.

In the natural defoliation treatment, root starch concentration declined prior to bloom with no associated rise in soluble carbohydrate in the root. This suggests that this reserve may be being utilised within the roots for respiration, metabolism and growth, lost through root exudates, or exported to other sinks such as the early stages of flowering and shoot growth.

The rapid increase in sorbitol concentration in roots from one week before full bloom, immediately following the pre-bloom root starch decline, cannot be attributed to remobilisation of starch as starch concentration either remains unchanged or increases during this period. In spurs, a small decline in starch concentration between one week before bloom and two weeks after bloom cannot explain the rapid and protracted increase in sorbitol concentration from one week before full bloom. The timing of the start of sorbitol concentration increase in these structures corresponds closely to the period when primary rosette spur leaves begin exporting carbohydrates to other structures (mostly within the spur) (Tustin and Lai, 1990). Therefore, it is likely that the rise in sorbitol concentration is indicative of a change in carbohydrate supply from stored reserves to current season's photosynthesis. It is only after this rise, from 2 - 4 weeks after bloom, that carbohydrate limitations are thought to have the greatest impact on fruit set (Lakso et al., 1998; Lakso et al., 1999). These results suggest that the use of stored carbohydrates had declined, and export of photosynthates from new leaves had become the major carbon source for growth, prior to the period where carbohydrate limitation is thought to affect fruit set. Furthermore, increase in concentration of starch in all structures from two weeks after full bloom, suggests that other sinks such as storage sinks compete effectively for carbohydrates at this time (de Jong, 2014).

ASE, greatly reduced bud numbers on the same trees during the two previous seasons, but this had no effect on carbohydrate concentrations in any structure at any date during the carbohydrate sampling period. In the season reported on here, total floral bud density in the PFD treatment was 11.6 buds.cm⁻² BCA (S+T + lateral). ASE successfully reduced the number of sinks in the canopy,

through a ~50% reduction in floral bud density (5.9 buds.cm⁻² BCA, lateral buds removed) and resulted in a 38% increase in fruit set within individual buds. These responses could support the hypothesis that reserve carbohydrates contribute to fruit set, because reducing the number of competing sinks prior to budbreak, may increase the availability of carbohydrate reserves to remaining sinks, leading to increased within-bud fruit set.

Early spring growth is supported from reserve carbohydrates sourced through the whole tree (Priestley, 1964b; Hansen and Grauslund, 1973). Consequently, if reserve carbohydrates do contribute to fruit set, differences in reserve carbohydrate concentration in shoots, spurs and roots would be expected to affect fruit set. In this study, early defoliation resulted in a reduction in winter total non-structural carbohydrate concentration of 45% in roots and 10% in shoots, but this had no effect on fruit set. Although winter total non-structural carbohydrate and starch concentrations were reduced in the early defoliation treatment, soluble carbohydrate concentration (especially of the transport carbohydrate sorbitol) generally did not differ between defoliation treatments. As sorbitol plays a critical role in establishing young fruit as sinks during fruit set (Archbold et al., 2011), fruit set may have been unaffected by low total non-structural carbohydrate concentration in trees defoliated early, because these trees may have been able to maintain sorbitol concentration independently of differences in starch concentration. Higher concentration of glucose + fructose and raffinose + stachyose in the early defoliation treatment prior to two weeks after bloom may have provided a source to maintain sorbitol concentration. However, as utilisation of these carbohydrates formed only a small part of total non-structural carbohydrate pool, this argument probably does not explain the fruit set response result sufficiently.

In research presented here, early-season trends of carbohydrate concentration in bark and wood of the permanent tree structure were not studied. Although these organs also supply reserve carbohydrates to sinks in early spring, they do not act very differently to organs such as those investigated (Priestley, 1964b; Hansen and Grauslund, 1973) and so are unlikely to contribute to the observed fruit set responses.

The lack of evidence for a role of reserve carbohydrates in fruit set agrees with Hennerty and Forshey (1972) who did not find any relationship between fruit set and spur reserve carbohydrates in 'Golden Delicious' at full bloom. Their work did show a positive relationship between the nitrogen content in

spurs or flower clusters and fruit set, suggesting that nitrogen limitation may have played a greater role in that study.

Reserve carbohydrates reach a minimum at about 2 – 4 weeks after bud break in young trees (Priestley, 1963; Kandiah, 1979b), and photosynthesis supplies significant portions of newly synthesised carbohydrates to fruit from about petal fall (Tustin and Lai, 1990; Corelli Grappadelli et al., 1994). This period precedes the stage 2 – 4 weeks after bloom, when carbohydrate supply limitation is thought to reduce fruit set (Lakso et al., 1998). Therefore, differences in within-bud fruit set between ASE and PFD treatments may be better explained by differences in the availability of current season's photosynthate rather than reserve carbohydrate. Altered leaf assimilation rate is unlikely to have played a role in this result because positive trends between crop load and leaf assimilation rate only occur from ~5.5 weeks after full bloom, after the critical period for fruit set determination, and do not generally occur at crop loads as high as those set in this investigation (Palmer et al., 1997). Investigation of canopy light interception using the trees in this study during the previous season showed that even though ASE treatment set at 6 buds.cm⁻² BCA removed ~60% of floral buds, canopy light interception was not reduced at any stage of the season, even as early as two weeks after bloom (Breen et al., in press). This response suggests that the carbon assimilation potential of canopies was not reduced in ASE canopies despite greatly reducing the number of buds. The resulting early-season reduced foliage density probably resulted in increased daily light exposure of leaves on individual spurs when ASE was imposed at this bud density. Increased photosynthesis is likely to benefit closely associated sinks most, such as fruit within a spur, and consequently improve within-bud fruit set, because spur and bourse shoot leaves primarily supply sinks within the spur structure (Tustin and Lai, 1990; Tustin et al., 1992; Corelli Grappadelli et al., 1994). Previous work comparing fruit set in trees treated with ASE, PFD or flower cluster thinning at pink bud stage has proposed a similar conclusion (Breen et al., 2015).

It has been suggested that a greater availability of carbohydrate reserves in apples prior to winter, or their utilisation over winter contributes to advance bud break, which may allow trees to achieve autotrophism earlier in the season (Greer and Wünsche, 2003). Delayed early-season phenology in trees where winter and early season carbohydrates were reduced or thought to have been reduced has also been observed in research by Faby and Naumann (1986) and Tustin et al. (1997). In results presented here, treatments with greater carbohydrate reserve concentration at dormancy showed

greater utilisation of these prior to flowering, and earlier flowering date, despite having the same date of bud break induced by a dormancy breaking spray. Greater reserve carbohydrate concentration may have provided increased substrate to enable more rapid canopy development early in the season.

A number of authors have found that growth in the current season is associated with reserve carbohydrate concentration. In mature 'Golden Delicious', Hennerty and Forshey (1972) found that growth on spurs at three weeks after bloom was significantly correlated with spur reserve carbohydrates at full bloom, but growth on shoots was not. However, growth on both spurs and shoots was also significantly correlated with spur soluble N at full bloom. Tustin et al. (1997) found that early defoliation reduced spur leaf area and trunk cross-sectional area. Other studies have also shown a positive relationship between shoot growth and reserve carbohydrates (Abusrewil and Larsen, 1981; Abusrewil et al., 1983) and yet this response does not always appear to be the case (Priestley, 1963). van Hooijdonk et al. (2010) showed that reducing bud density through the whole canopy in 'Scilate' (Envy™) using ASE, greatly reduced the proportion of spurs and increased the proportion of shoots that subsequently developed in the canopy. As with results from the current research, the majority of these shoots developed from lateral buds on current seasons floral bourses. In the results reported here a greater proportion of these buds extended to form short shoots, rather than spurs, in treatments where higher winter carbohydrate reserve concentration occurred (natural defoliation) together with lower numbers of competing sinks in spring (ASE). Greater numbers of competing sinks (PFD), or reduced winter carbohydrate reserve concentration (early defoliation), or both, reduced shoot density by 64% or more. There was some indication from the data that trees having both the greatest density of sinks (PFD) and lowest concentration of winter carbohydrate reserves (early defoliation) produced the lowest density of shoots, but this difference was not statistically significant. Large and rapid decrease in carbohydrate concentration over bud break and prior to full bloom in trees where winter carbohydrate concentration was greater (naturally defoliated trees) suggests that stored carbohydrate may have been utilised in the early stages of development of the new season's canopy. In floral spurs, rapid development of floral components, differentiation of conducting tissue and early growth of the vegetative meristems (lateral buds subtending the floral meristem) occur during this period (Bergh, 1985; Jackson, 2003). Greater availability of carbohydrates prior to full bloom in the ASE + natural defoliation treatment may have allowed

improved early floral and vegetative development, either by direct supply of carbohydrates to these meristems, or more indirectly, for example through increased root development over late winter and in early spring (Priestley, 1964a). Early gains in vegetative development may lead to increased photosynthetic potential through greater leaf area. Increased proportions of shoots observed in the ASE + natural defoliation treatment by the end of the season are likely the response to this improved development. In contrast, unlike the vegetative growth response, the fruit set response to thinning and defoliation appeared to be strongly regulated by availability of current season's photosynthate, not stored carbohydrates.

Conclusions

A prolonged period between harvest and autumn leaf fall, such as occurs naturally in 'Royal Gala' grown in New Zealand, established a high concentration of stored carbohydrate reserves in the subsequent winter. These reserves appeared to play an important role in metabolic function of the trees early the following season, because concentration of total non-structural carbohydrates declined by about one third between dormancy and one week before full bloom the following spring. When within-bud fruit set in PFD trees was compared with that in ASE trees, results appeared to support a strong role for utilisation of re-mobilised stored carbohydrates in fruit set, as the 38% increase observed in within-bud fruit set in ASE trees may be explained by the greatly reduced sink density in ASE in the early season. However, lack of differences in fruit set between defoliation treatments, despite differences of up to 45% in winter total non-structural carbohydrates, challenged the hypothesis that stored carbohydrates are important in fruit set.

In spurs, concentration of total non-structural carbohydrates, and especially sorbitol, increased early, from one week before full bloom onwards. This timing corresponds with the time that newly expanded spur leaves begin to export carbohydrates to other sinks within the spur, and before the period two to four weeks after bloom, during which carbohydrate limitation is thought to reduce fruit set. Therefore, we conclude that supply of newly synthesised carbohydrates plays a greater role in determining fruit set than remobilised, stored carbohydrates.

Previous studies (Breen et al., in press) have shown that thinning of buds to a density of 6 buds.cm⁻² BCA using the ASE process did not reduce whole canopy fractional light interception, despite reducing floral bud density by about 60%. It is thought that a consequence of this is that leaves on

individual spurs have increased exposure to light, increasing photosynthesis and carbohydrate availability to developing fruit within the spur, and consequently improving within-bud fruit set. This hypothesis offers an explanation for the observed increase in within-bud fruit set in ASE trees compared with PFD other than increased access to reserve carbohydrates.

In trees where a greater concentration of non-structural carbohydrates were utilised between dormancy and full bloom (natural defoliation), and fewer competing sinks were present in spring (ASE), more buds extended to form shoots during the season. Therefore it is likely that stored carbohydrates have some direct role in initial development of floral spurs, such as early growth of floral components and development of the vegetative bourse bud subtending the floral meristem, but do not appear to play a great role in fruit set.

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Chapter 6. Summary and implications

Introduction and thesis hypotheses

Although the Australian and New Zealand apple industries differ markedly in productivity and primary target markets, producers in both nations seek to improve fruit quality and production in order to increase export earnings. Naturally occurring high floral density and variability in flowering and fruit set on commercial orchards is discordant with this goal as they cause variable fruit quality and yield, and result in reduced production efficiency (O'Rourke, 2015). Current management tools used to reduce excessive flower and fruit numbers rely on a series of applications of chemical thinners over flowering and fruit set, followed by a final hand thinning after final fruit drop. Although this practice is in widespread commercial use, growers often find results unsatisfactory because responses are unpredictable, and delays in removal of excess fruit result in loss of potential fruit size (and therefore yield) and fruit quality. These challenges are not limited to Australia and New Zealand, but face commercial apple producers throughout the world. Improved understanding of the physiology of flowering and fruit set in apple is essential to develop technology that will support industry targets of improved fruit quality and yield. Precise management of floral and vegetative bud numbers and ratios in whole tree canopies at bud break offers a mechanism to investigate early season carbohydrate source-sink relationships and their effect on seasonal development of the tree and crop. Artificial spur extinction (ASE) provided a tool with which to do this. In this research, I investigated two hypotheses:

- 1) Reducing the number of floral and vegetative sinks early in the season will reduce competition among remaining sinks for stored reserves and newly synthesised photosynthates, and result in increased fruit set.
- 2) Removal of whole buds, for example through ASE, will result in impaired fruit set because reduced early season spur leaf area (carbohydrate source) will reduce light interception by the canopy.

Summary of research

Chapter 2: Effects of environment and floral intensity on fruit set behaviour and annual flowering in apple

Flowering intensity of 'Gala' trees was manipulated using ASE on five sites through New Zealand and Australia over four seasons. Investigating a range of branch bud densities and naturally occurring

differences in weather and orchard environment among regions and seasons allowed investigation of the effect of floral intensity and environment on fruit set and return annual flowering. Flowering and fruit set in Control treatments, especially at high floral bud densities, was typical of commercial trees; highly variable, highly responsive to environmental conditions prevalent during fruit set, and largely unresponsive to differences in floral bud density. Reduction of floral bud density using ASE greatly reduced variability in fruit set and flowering and allowed predictive models to be developed for each site describing the response of fruit set to floral bud density. The physiology affecting these responses was investigated and is reported on in Chapters 3 to 5.

Chapter 3: Method of manipulating floral bud density affects fruit set responses in apple

Research in this Chapter was undertaken to investigate the influence of early season carbohydrate source size (specifically leaf area and access to stored carbohydrates) and total sink demand (specifically floral and vegetative bud density) on fruit set and components of yield. Artificial spur extinction (ASE) was used as a tool to manipulate sink size (and therefore total sink demand) through complete removal of whole buds during dormancy. This was likely to have reduced early season leaf area while improving access of remaining sinks to stored carbohydrates. Flower cluster thinning (FCT) prior to bloom reduced floral sink size without altering vegetative sink size or leaf area. These treatments were compared with a Control, where fruit numbers were not modified until after final fruit drop when crop loads on all treatments were set. Greater within-bud fruit set and mean fruit weight in FCT and ASE treatments suggested that reduced sink number in FCT and ASE treatments increased carbon availability within remaining floral spurs during early-season development compared with the Control. The process by which this occurred appeared to differ between FCT and ASE. In FCT, reducing the density of flower clusters (sink size) but not leaf area may have increased carbon availability to remaining sinks, improving fruit set and development. In ASE removal of whole buds reduced floral density, but also reduced early season photosynthetic potential through removal of leaf area. Increased availability of stored reserves to remaining sinks in ASE cannot explain this response because mean fruit set per bud in FCT was generally greater than in ASE, and FCT was applied later, at 'pink bud' stage when the contribution of stored reserves is considered to be largely complete. In ASE, removal and uniform spatial distribution of buds may have improved irradiance of fruiting spurs, thereby increasing photosynthate availability to developing fruit within the spur. These conclusions

were investigated in Chapters four and five where the development of whole canopy light interception and the role of stored carbohydrates in ASE canopies are discussed.

Chapter 4: Artificial Spur Extinction Alters Light Interception by 'Royal Gala' Apple Trees

Increased within-bud fruit set and improved fruit development observed in trees managed using ASE suggests that when early season bud density is reduced using ASE, carbon availability to remaining floral spurs is increased. However, because ASE greatly reduces total bud numbers on the tree it is likely to reduce total early-season leaf area and thus reduce light interception and photosynthetic potential. To investigate this response in ASE, a series of bud densities were imposed on 'Royal Gala' canopies in a commercial block in New Zealand and compared with unmodified trees.

Fractional light interception was measured at intervals from shortly after bud break, through one whole season. Before petal fall, at ASE bud densities of 4 and 6 buds.cm⁻² branch basal cross-sectional area, which were required in order to achieve commercial crop loads, early season light interception was either not affected (ASE6), or only slightly reduced (~2%, ASE4) compared with unmodified trees. During most of the season, light interception by ASE canopies was greater than unmodified canopies. Greater development of bourses and bourse shoots on ASE canopies probably increased light interception by these shoots which are closely associated with flowers and developing fruit, increasing harvest mean fruit weight.

Chapter 5: A re-evaluation of the role of carbohydrate reserves in fruit set and early season growth of apple

Carbohydrate reserves are considered essential in supporting early spring growth, however their contribution to fruit set is unclear. In canopies where ASE was imposed, reduced competition among developing buds for limited carbohydrate reserves may contribute to an increase in within-bud fruit set compared with unmodified canopies. In ASE and unmodified trees with high yields, carbohydrate reserve concentration in winter was manipulated through early defoliation shortly after harvest in the previous season and compared with carbohydrate reserve concentration in trees where natural defoliation occurred. Carbohydrate concentration in roots, shoots and spurs was determined the following season from dormancy through to just after final fruit set. Results showed that differences in reserve carbohydrate concentration when growth resumed in spring had no direct influence on fruit

set, and fruit set appeared to be more reliant on the availability of newly synthesised photosynthates. However, bourse shoot development was enhanced in treatments which had unmodified reserve concentration and reduced sink demand induced by ASE. Stored carbohydrates appeared to be utilised in the very early development of vegetative sinks and may promote canopy development in spring.

General Discussion

In all three experiments where fruit set was investigated (Chapters 2, 3 and 5), removal of whole buds shortly before bud break using ASE, increased the numbers of fruit that set within remaining floral buds compared with trees that were left unmodified until final fruit drop was completed (Control / Unmodified trees). When a series of ASE bud densities were examined (Chapters 2 and 3), the greater the number of buds removed, the greater the number of fruit that set within remaining individual buds. Generally, this was because fewer floral buds set zero fruit and more buds set two or more fruit. When only floral carbohydrate sink numbers were reduced (FCT) instead of reducing both floral sink and leaf (source) numbers (ASE), fruit set responses were only slightly amplified. Compared with Control / Unmodified trees, ASE treatments produced fruit of 17 – 32g greater final mean fruit weight (Chapters 3 and 4), but differences in the fruit quality attributes flesh firmness and dry matter content did not show consistent differences.

These results are consistent with the first hypothesis that reducing the number of sinks early in the season will reduce competition among remaining sinks for available carbon from stored reserves and newly synthesised photosynthates, and enable an increase in fruit set. However, a number of considerations arise from the results.

- The effect of possible limitations in other essential resources such as nitrogen or minerals, which were not investigated in this research, cannot be excluded. Investigation of the contribution of these resources would greatly assist in interpretation of the physiology of fruit set in apple.
- The effect of reduced sink number on within-bud fruit set and final fruit weight was clear. However, fruit flesh firmness and dry matter content were not always altered. Increased final fruit weight may have been partly the consequence of greater access to early-season carbon resource supplying fruit cell division in the first few weeks after bloom

(Jackson, 2003) when competition among sinks is high. Deposition of cell wall material and influx of solutes is primarily dependent on the supply from current seasons' photosynthesis (Jackson, 2003) and is not limited to periods when carbohydrate resource supply is limited. Therefore, differences in flesh firmness and dry matter content may have been less distinct because canopy light interception (carbohydrate resource supply) did not differ greatly between ASE and Control / Unmodified treatments for much of the season (Chapter 4).

- The small differences in fruit set and final fruit weight between FCT and ASE treatments (Chapter 3) despite much reduced bud number and associated early season leaf area in ASE, led to the hypothesis (discussed below) that the mechanisms by which this occurred differed between the ASE and FCT treatments. This emphasised that, in particular, seasonal development of canopy light interception, and the role of stored vs newly synthesised carbohydrates in fruit set, required investigation.

The second hypothesis proposed in this research was that:

Removal of whole buds, for example through ASE, will result in impaired fruit set because reduced early season spur leaf area (carbohydrate source) will reduce light interception by the canopy.

At bud densities that are necessary to achieve commercial crop loads, ASE generally removed more than 50% of the spur + terminal floral buds, and often as much as 75%. Considering that not only floral buds were removed, in most situations the total proportion of buds removed would be greater than this. Where data for the total bud densities present in winter on Control / Unmodified trees was collected (15.9 buds.cm⁻² BCA, Chapter 5), if ASE was imposed at a commercial bud density of 5 buds.cm⁻² BCA, 69% of Spur + Terminal buds would have been removed. Despite removal of this large proportion of buds and their associated spur leaf area, fruit set on remaining floral buds (Chapters 2, 3 and 5) and final fruit weight (Chapters 3 and 4) were increased over Control / Unmodified trees. These results do not support the second hypothesis. Results in Chapter 3 showed that greater fruit set and improved components of yield are unlikely to be the result of improved access to remobilised stored reserves in ASE, because FCT was imposed after the period when most reserves are likely to have been metabolised, and yet had similar fruit set and final fruit weight to ASE. The results of the investigation into seasonal canopy light interception (Chapter 4) give valuable

insight into canopy development in ASE trees and provide an alternative hypothesis as to a mechanism for increased fruit set and development. This experiment revealed that despite removal of the large numbers of buds discussed above, at bud densities required for commercial yields, whole canopy light interception was either not reduced (ASE6) or reduced by very small margins (ASE4, 2%) and only transiently, until 4 WABB. Furthermore, this timing was well before the period where carbohydrate limitation is regarded to reduce fruit set (Greene et al., 2005; Lakso et al., 2006). For much of the season, light interception of ASE canopies was slightly (3 – 4%) greater than in Control / Unmodified trees. This suggests that carbon assimilation potential of canopies is maximised at the leaf area associated with bud densities as low as 4 – 6 buds.cm⁻² BCA. The study presented in Chapter 5 supports this conclusion because comparing ASE with Control / Unmodified trees, no differences were found in the concentrations of carbohydrates in any structure during the winter after two consecutive seasons where treatments at bud densities of 6 buds.cm⁻² BCA yielded in excess of 100t.ha⁻¹ fruit. At natural bud densities (such as those on Control / Unmodified trees), it is likely that developing foliage creates considerable shading among leaves even very early in the season. Because ASE not only removes buds but also distributes buds evenly along branches, ASE managed canopies may reduce shading among leaves and increase light penetration into the canopy. In this situation, individual fruit bearing spurs may be exposed to an improved light environment from the beginning of the season, with individual leaves having greater daily duration of light exposure. Although increased leaf assimilation rate does not generally occur at crop loads as high as those used in this research (Palmer et al., 1997), potential photosynthesis is greater in leaves that expand in high light environments (Flore and Lakso, 1989). Results from research in Chapter 5 also suggest that reducing the number of floral buds may improve the availability of carbohydrate reserves to vegetative sinks early in the season. Aside from increasing shoot growth, these reserves may also increase leaf area of individual leaves. Improved light environment and photosynthetic potential in ASE managed canopies may compensate for reduced total leaf area imposed through bud removal and enable increased fruit set on remaining buds and increased final fruit size. A detailed study of individual spur daily irradiance patterns and investigation of net photosynthesis on an individual leaf basis and whole canopy gas exchange on ASE and Control / Unmodified canopies would give further insight into this response.

The role of remobilised carbohydrate reserves in fruit set appears to be limited, or at least indirect. In Chapter 5, reducing floral sink number by about 50% using ASE increased within-bud fruit set by ~38%, however, reducing reserve carbohydrate concentration considerably (10 – 60 %) had no effect on fruit set. Rapid replenishment of carbohydrate content of root, shoot and spur tissue commenced from about 3 weeks before the period during which carbohydrate limitation is thought to reduce fruit set (two to four weeks after bloom). Interestingly, these carbohydrates also appear to begin to replenish storage reserves from as early as 2 WAFB, the beginning of the carbohydrate limitation period that affects fruit set. This indicates that the use of reserves for early season growth is complete prior to the period of carbohydrate limitation, and so fruit set is likely to be more dependent on current season photosynthate. Replenishment of carbohydrate storage reserves from the beginning of the period when carbohydrate demand is high and carbohydrate limitation is thought to occur (2 WAFB) is interesting as reserve sinks are often regarded as relatively low priority. As this response was observed equally in both ASE managed trees (where all bourses and bourse shoots held developing fruit) and unmodified trees (where vegetative spurs occurred), it appears that the same bourses that hold developing fruit may supply carbon for replenishment of storage reserves from as early as full bloom. This result does not necessarily challenge the view that carbohydrate limitation occurred from two to four weeks after bloom, but does support the view that storage sinks are active sinks and are not merely replenished during times when current supply exceeds demand from other sinks (de Jong, 2014).

Aside from increased within-bud fruit set, improved light ingress within the canopy may have other advantages not investigated in this study such as improved fruit colour (van Hooijdonk et al., 2014) and improved floral bud formation in the interior of the tree (Cain, 1973). In New Zealand and Australia, I have frequently observed trees in mature orchards on dwarfing rootstocks having more than 10 limbs per meter of effective canopy height, compared with the 6 – 7 calculated to be required to optimise canopy structure prior to using ASE. In orchards with this high number of limbs the productive canopy moves outwards from the trunk, because the few buds present inside the canopy are usually not floral. Fruit that recedes into this area of the canopy over the season as fruit weight increases (which may be a significant proportion commercially) does not colour well, and as the productive canopy encroaches into the orchard alley, movement becomes difficult and fruit is exposed to mechanical damage. These features demonstrate that large numbers of branches have no

practical value or benefit to the orchard system beyond the minimum branch number needed to achieve maximum light interception. Canopies with fewer limbs and buds are also likely to be more porous which may improve coverage of orchard sprays and reduce duration of leaf wetness, reducing fungal infection (Simon et al., 2006).

Commercial orchard managers frequently view prolific flowering and fruit set as a first step in assuring a profitable crop and as an insurance against events that may cause fruit drop or early season fruitlet damage (e.g. hail). In New Zealand, I have observed that 'Gala' trees grown on dwarfing rootstocks often produce 3000 – 4000 flowers where only ~250 fruit per tree are required. One of the strongest considerations in the view of growers is that the outcome of application of thinning sprays is unpredictable. For this reason, growers often prefer to keep many branches and buds, thinking that this equates with many flowers. As discussed above, this is not necessarily the case. To some extent, the view that some form of biological 'insurance' is needed in commercial production was supported by the examination of natural flowering and fruit set (Chapters 2 and 3), because fruit set was highly variable, generally unresponsive to natural differences in flowering intensity, and highly responsive to weather and other environmental conditions.

Development of regular annual flowering through induction of 'bourse-over-bourse' behaviour in trees subjected to the ASE process is well documented (Lauri and Lespinasse, 1993; Lauri et al., 1997; Lauri and Lespinasse, 2000). Although the 'bourse-over-bourse' process was not specifically recorded in this thesis, results presented show that ASE greatly increased the consistency of flowering (reduced standard deviation of floral bud density) among seasons compared with Control / Unmodified trees across all sites tested (Chapter 2). However, ASE did not only reduce variability in floral bud density among seasons, but also among trees and sites. The bourse-over-bourse phenomenon is observed more strongly in genotypes where bourse buds extend to form shoots or 'dards' (Lauri and Lespinasse, 1993). 'Gala' is regarded as a type III cultivar. In this genotype, natural growth results in stronger branching than spur types (type II) but does exhibit development of the bourse-over-bourse characteristic as strongly as in type IV (Lauri et al., 1997). However, there are exceptions to the rule, such as 'Fuji', a type IV genotype which does not reliably express the bourse-over-bourse characteristic (Lauri et al., 1997). Work in this thesis shows that ASE increases the proportion of shoots produced in 'Gala' trees (Chapter 5). This may have initiated the bourse-over-bourse trait in this type III cultivar, resulting in increased regularity of flowering and indicating a

certain plasticity of response to methods of crop management. Lauri and Grappadelli (2014) have suggested that genotypes differ in the plasticity of their response to management techniques such as centrifugal training (which uses ASE). It would be of considerable interest from both the physiological and commercial perspective to validate differences in plasticity of response among commercial genotypes, especially those considered to be prone to a highly irregular flowering habit, such as 'Sciros' (Pacific Rose™).

This thesis shows that if ASE is used, the prevalent commercial view that prolific flowering and fruit set are required to ensure sufficient fruit for a commercial crop is invalid. Ironically, it appears that this view actually reduces consistency of flowering and fruit set and has potential to reduce fruit size. Regulating limb numbers to 6 – 7 per m of effective canopy height and reducing bud numbers using ASE optimised tree structure for light interception, improved predictability of flowering and, because fewer floral buds set zero fruit, improved the predictability and reliability of fruit set. Greater predictability in flowering and fruit set allowed models to be developed that described the response of fruit set to floral bud density within each orchard. Although there were similarities in response between some orchards, there did not appear to be single model that accurately described the response of the 'Gala' genotype across diverse environments. This may be because regional weather and individual orchard management and environment play a large role in flowering and fruit set. However, within sites, the models developed were able to describe altered fruit set responses with a high level of accuracy (67 – 90%) when seasonal differences in weather and environment occurred. Predictive fruit set models have previously been produced for 'Scifresh' (Jazz™) from one site (Tustin et al., 2012). If the full value of this technology is to be gained commercially, further development of the responses of additional cultivars to ASE including their fruit set models would be beneficial.

Conclusions

A number of over-arching conclusions may be made from results of this research which add to our knowledge from other studies.

In apples, carbohydrates may be highly mobile through the tree. In the early season, before new leaves expand and net carbon exchange becomes positive, active meristems such as developing buds can access stored carbohydrates from sinks which are often spatially distant, such as roots.

Soon after new leaves develop, until leaf fall, carbohydrate reserves in roots and other organs are replenished from leaves developed from these meristems.

However, from around full bloom, developing fruit sinks appear to be highly dependent on carbohydrates supplied by leaves situated in close proximity, such as those subtended on the same spur structure. If leaves of spurs that have developing fruit are able to intercept a high proportion of incident light (for example trees where ASE has been applied), additional leaves, such as those on neighbouring vegetative spurs do not improve fruit set or final fruit size and quality appreciably.

This has significant implications for management because whole canopy light interception, and therefore fruit set, yield and fruit quality is maximised at branch and bud densities very much lower than most orchard managers currently understand and use. With 'Royal Gala', additional branches and buds that do not bear flowers or fruit destined for final harvest appear to be not only unnecessary to optimising fruit set, yield and fruit quality, but they may be detrimental, increasing uncertainty of fruit set, reducing spray penetration, and reducing fruit colour through reduced light penetration into the canopy.

For orchard management, one of the very useful aspects of the way in which fruit set was increased on ASE and FCT treatments (where floral sink number was reduced before flowering), was that few floral buds set zero fruit. This result occurred consistently among seasons (ASE and FCT) as well as among sites (ASE). Practically, this result means that the fruit set response became highly predictable. At floral bud densities set to achieve commercial crop loads with fruit held as a single fruit per bud, in ASE and FCT, selected buds were highly likely to set fruit. Consequently, from a canopy management point of view, any buds that are not required to hold fruit destined to final harvest may be removed.

In ASE management, the combination of advantages gained from reduced bud density and very early thinning shows that this precision thinning method may provide a highly effective approach to crop load management, producing highly predictable results without the need for chemical thinning. This contrasts strongly with current commercial practices. Further research in this area has the potential to improve our understanding of whole tree physiology and commercial orchard management in apple, and this may provide a model for use in other crops.

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